Genetic Variation In Insect Performance In A Tritrophic Interaction On Wild And Cultivated Beans.

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Genetic variation in insect performance in a tri-trophic interaction on wild and cultivated beans

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SUMMARY:

The process of domestication often alters the chemical composition and nutrient contents of plants. When plant quality is modified, insects feeding on these plants and the natural enemies of these insects have to adapt to these changes. Thus, variation in the first trophic level may largely determine the type and evolution of the interaction between parasitoids and their phytophagous hosts. This study examined the consequences of the bean domestication in *Phaseolus vulgaris* for the interactions between phytophagous insects and their natural enemies. *Zabrotes subfasciatus* (Coleoptera: Bruchidae) feeds on seeds of the genus *Phaseolus* throughout Mexico and Central America. The parasitic wasps, *Stenocorse bruchivora* (Hym.: Braconidae) and *Dinarmus basalis* (Hym.: Pteromalidae), are the main natural enemies of *Z. subfasciatus*.

I investigated the influence of environmental (cultivated vs. wild seeds) and genetic components on the behaviour and performance of *Z. subfasciatus* and two parasitoid species that differ in their degree of host specificity.

For the bruchid, results showed that the oviposition behaviour and fitness components are affected by the quality of the seeds of *P. vulgaris*. I also found differences between bruchid families, which argues for a strong genetic component.

Both parasitoid species were affected by the quality of the seeds on which their hosts were reared. This effect was stronger for the specialist: *S. bruchivora* was more selective when choosing a host. In contrast, the behaviour of the generalist *D. basalis* was more affected by the host availability.

Transplant experiments using wild populations of *S. bruchivora* also showed a strong genetic component for the parasitism behaviour and fitness traits, and great variation among parasitoid populations. It appears however that this variation in performance among

populations is decreased when parasitoids are exposed to cultivated seeds, which themselves present a more homogeneous resource for the insects.

RÉSUMÉ:

Très souvent, la domestication d'une plante change sa composition chimique et nutritionnelle. Lorsque la qualité de la plante est modifiée, les insectes qui se nourrissent de cette plante doivent s'adapter à de tels changements. Dès lors, une variation dans ce premier niveau trophique détermine le type et l'évolution des interactions entre des guêpes parasites et leurs hôtes phytophages. Cette étude porte sur les conséquences de la domestication du haricot *Phaseolus vulgaris* sur les interactions entre un insecte phytophage et ses ennemis naturels. *Zabrotes subfasciatus* (Coleoptera: Bruchidae) vit au Mexique et en Amérique Centrale où il se nourrit des graines de haricots. Les guêpes parasites *Stenocorse bruchivora* (Hymenoptera: Braconidae) et *Dinarmus basalis* (Hymenoptera: Pteromalidae) sont les principaux ennemis naturels de *Z. subfasciatus*, mais diffèrent dans leur degré de spécificité en hôte.

Ce travail s'intéresse à l'influence des facteurs environnementaux (telle que la variabilité de la plante hôte suite à sa domestication: graines cultivées / graines sauvages) et génétiques sur le comportement et les performances du phytophage et des deux espèces de parasitoïde.

Pour le coléoptère, les résultats montrent que le comportement d'oviposition ainsi que les paramètres de fitness sont affectés par la qualité des graines de *P. vulgaris*. Il existe également des différences entre les lignées de bruches, témoignage d'une variabilité génétique.

Les deux espèces de parasitoïde sont également affectées par la qualité des graines sur lesquelles leurs hôtes sont élevés. Cet effet est plus marqué chez le spécialiste *S. bruchivora*, plus sélectif dans le choix de l'hôte, contrairement au généraliste *D. basalis* dont le comportement est beaucoup plus affecté par la disponibilité en hôte.

Des expériences de transfert faites avec trois populations sauvages de *S. bruchivora* ont également démontré une forte composante génétique dans le comportement de parasitisme et les caractéristiques de fitness. A cette composante génétique s'associe des variations entre les populations. Cependant, il apparaît que ces variations de performances entre les populations diminuent lorsque les parasitoïdes sont issus d'hôtes offerts dans des graines cultivées: celles-ci représentant une ressource plus homogène pour les insectes que les graines sauvages.

GENERAL INTRODUCTION :

I. Tritrophic system:

A tritrophic system is composed of three actors linked to each other through feeding relations. The first trophic level is the plant, which is used as a food source by a phytophagous insect that may attack any structure of the plant. These insects may themselves experience predation and parasitism by other insects, in particular parasitic wasps. Parasitoids lay their eggs on or within the bodies of other species of insects, and their larvae develop by feeding on the host causing its eventual death (Godfray, 1994). Direct and indirect interactions, occur between subsequent trophic levels. For example, between the plant and the phytophagous insect that feeds on the plant tissues, and between the insect and the parasitoid that attacks it.

a) Examples of relation between the first and second trophic level, and between the second and third trophic level:

The plant can defend itself against herbivores through toxins, repellents and digestibility reducers (Rosenthal & Berenbaum, 1992; Fritz & Simms, 1992). The interrelationships between these two levels are so strong that they may result in functional adaptations (coevolution) between the plant and the herbivore such as, in the case of bean plants and bruchid beetles (review in Center & Johnson, 1974). For example, in response to seed toxicity, the beetles develop resistance towards seeds or avoid the toxins by developing tolerance mechanism as it is the case for *Zabrotes subfasciatus* who has developed tolerance

for the proteinous alpha-amylase inhibitor contained in seeds of *Phaseolus vulgaris* (Ishimoto & Kitamura, 1992)

Between the second and third trophic level, there also exist strong interactions. A parasitic wasp has to locate the host and to evaluate its suitability. In the process, it may use cues that are emitted directly from the host. The host has evolved mechanisms to escape parasitism, for example by developing physiological responses (e.g. encapsulating the parasitoid egg, Carton & Kitano, 1981) or behavioural responses, such as, the leafminer *Phyllonorycter malella* which creates a heterogeneous environment by leaving a central area of uneaten tissue which acts as a protecting shield against parasitoids (Djemai *et al.*, 2000). Interactions between members of the same trophic level and between members of the first and third trophic level are also common (Price *et al.,* 1980; Dicke, 1994/1995; Monge *et al.,* 1995; Turlings & Benrey, 1998). Two parasitoid species can show different competitive abilities when faced with a parasitized host. For example, when *D. basalis* females are introduced on a host patch 24h after the parasitoid *Eupelmus vuilleti* (Eupelmidae), multiparasitism is low, and *D. basalis* females have a low fecundity. In the reverse case, the presence of hosts previously parasitized by *D. basalis*, increases the reproduction of *E. vuilleti*, which are able to kill the *D. basalis* eggs or larvae (Leveque *et al.,* 1993). Moreover one herbivore species can affect other tritrophic interactions on the same plant. For example, the presence of a non host herbivore (*Pieris rapae*) decreased the time spent searching by *Cotesia plutellae* on a plant infested by its host (*Plutella xylostella*), modifying the chemical information involved in these interactions (Shiojiri *et al.,* 2001).

b) Examples of interactions between the first and third trophic levels:

In response to the attack of phytophagous insects, the plants produce volatiles used by parasitic wasps as cues to locate their hosts (Turlings *et al.,* 1991; Vet & Dicke, 1992; Geervliet *et al.,* 1996). Because the plant odour is more easily perceptible, but less accurate than host odour (larval compounds, frass), parasitoid females use associative learning to link the presence of the host with specific compounds released by a damaged plant (Vet *et al*., 1995). In some cases, the naive parasitoid females are attracted by plant odours without reliable indication of host presence (Campan *et al.*, in press).

Not only plant chemicals can affect the third trophic level. The plant's genotype and its structure also affect the interaction between the herbivore and parasitic wasps (Fritz *et al*., 1997; Gingras *et al.,* 2002). For example, the higher survival of the leafminer *Phyllonorycter salicifoliella* appeared to be the result of significantly lower parasitism by eulophid parasitoids on hybrid plants (*Salix* spp.) compared to the parental species (Fritz *et al*., 1997). Gingras *et al*., (2002) investigated the structure of the plant, and using three dimensional artificial plants (varying in size, heterogeneity and abundance of connections between plant parts) on which *Ephestia kuehniella* eggs were placed and offered as hosts for *Trichogramma evanescens* females, showed variability in parasitism rate, indicating that females parasitoids were more efficient at finding host eggs when foraging on simple plant structure.

Some authors have suggested a mutualistic interaction between the first and the third trophic level. Both the plant that attracts the parasitoids, and the parasitoids that respond to the plant volatiles benefit by this type of interaction: the parasitoid is lured to a host for its reproduction, and the plant suffers less feeding damage from parasitized hosts. It is the case for the plant *Arabidopsis thaliana*: when *Pieris rapae* larvae are parasitized by *Cotesia rubecula*, there is not a significant reduction in seed production in comparison to undamaged

plants, whereas herbivory by unparasitized caterpillars results in a reduced seed production (Van Loon *et al.,* 2000). The plant can also benefit a parasitoid by providing nectar sources. Females of the parasitoid *Diadegma insulare* lived longer and their fecundity was increased in the presence of wildflowers that provided nectar (Idris & Grafius, 1995). In contrast, African farmers use in storage systems the leaves of *Boscia senegalensis* because they release methylisothiocyanate (MITC) which is toxic for the bruchid pest *Callosobruchus maculatus* reducing also the density of the parasitoid *D. basalis* (Sanon *et al.,* 2002).

c) Modification of these interactions due to the domestication of the plant by humans.

Not only the insects use plants as a food resource. Humans due to the development of agriculture, have a strong impact on the plants mainly by their domestication. Depending on its human use, a plant can be selected in two different evolutionary paths: one as a vegetable form, and one as a seed form. The size of fruits and seeds are increased from the wild plant, and represent a better resource with a higher protein content, as is the case for seeds of the genus *Phaseolus* (Evans, 1993). While the seed quality increases in domesticated plants, the level of chemical defensive compounds decreases (Rhoades, 1979; Rosenthal & Dirzo, 1997). These differences in the general composition between wild and cultivated plants induces changes in the fitness of the herbivores and the parasitoids associated with them (Ishimoto *et al.,* 1995; Idris & Grafius, 1996). For example, the parasitoids *Cotesia glomerata* and *Stenocorse bruchivora* that emerged from hosts (*Pieris rapae* and *Zabrotes subfasciatus*) reared on higher quality plant (cultivated forms: *Brassica oleracea* and *Phaseolus coccineus coccineus*), had a shorter development time and higher survival than parasitoids that emerged from hosts on suboptimal plants (wild forms: *Lunaria annua* and *P. c. formosus*), (Benrey *et al*., 1998).

The last source of variation in the interactions between the different actors of a tritrophic system is the genotype of the insects. Due to fragmentation of suitable habitats (in this case the presence of the host plant), isolation by distance or presence of geographical barriers, genetic subdivision may arise through restriction in gene flow between insect populations (e.g. Gonzalez *et al.*, 2000). This process can be the origin for behavioural and performance variations between populations (Pack & Van Heinigen, 1985; Fox, 1993; Fox *et al*., 1994; Perez-Maluf *et al*., 1998) and in extreme cases, for local adaptation between an insect and its host (Mopper & Strauss, 1998).

Tritrophic interactions are complex systems, each level interacting with the others, and members of one level influencing each other. My PhD work, focused on the effects of plant variation as a result of plant domestication, in a tritrophic interaction. The studied model is composed of *Phaseolus vulgaris* (Leguminosae), domesticated as a food resource in Central America from 7500 BC, one of its main herbivores *Zabrotes subfasciatus* (Coleoptera) that attacks the seeds, and two parasitoid species, *Stenocorse bruchivora* (Hymenoptera: Braconidae) endemic to America and *Dinarmus basalis* (Hymenoptera: Pteromalidae) originated from Africa. I wanted to demonstrate that plant quality affects both the second and third trophic levels (behaviour and performance), but also that there is variation in these effects in relation to the life history and the diet specificity of the insects involved in this interaction.

This system is suited to test the effects of the plant domestication on the relations between the second and third trophic level, for several reasons. The bean *P. vulgaris* is an important crop worldwide used, with maize, as stapled diet of populations in Latin America. Its domestication story and phylogeny has been well studied (see next part). It is easy to find

wild and cultivated forms in Mexico, and easy to use them for experiments. Moreover, both the bruchid and the parasitoids are easily reared in laboratory and manipulated for behavioural tests.

II. Plant domestication:

A plant is called “domesticated” when it has been modified from its wild form (Simmonds, 1979). Domestication is "an adaptation of the initial wild or weed plant to cultivation on plantations" (Kupzow, 1980). According to this author, there are two main types of adaptation to new environmental conditions: the first one is a result of successful growth and development without genetic change of the plant ("naturalization"), and the second one requires genetic transmutation ("acclimatization"), depending on the easiness to be cultivated and domesticated. Plant domestication is a strong selection which leads to different varieties, plant phenotypes with their own characteristics, very different from the wild form, which is called the “original form”. For Evans (1993) based on Johannessen's definition, the domestication refers obligatorily to genetic changes which are not a single event but a continuing evolutionary process beginning with cultivation.

Domestication of wild plants is due essentially to the development of agriculture from the end of the 19th century (Kupzow, 1980). But the origin of domestication of some plant species occurred several years B.C. For example: barley (9000 B.C.), rice (5000 B.C.), maize (7000 B.C.), and beans (7500 B.C.) (review in van Raamsdonk, 1993). The main selection takes place for economic reasons such as, better food quality and productivity of edible parts for fruits and vegetables or for ornamental plants. Depending on the human use, a plant can be selected in two different evolutionary paths: as a vegetable form, and as a seed form. Such is the case of *Amaranthus spp*. an important food resource for Mexican populations (Mapes *et*

al., 1996). The vegetable forms are semi cultivated with a delay in the development of inflorescences and produce a high proportion of leaves over extended periods of time. In contrast the "grain-producing" amaranths are cultivated and selected to have a well developed central inflorescence. In the case of a nutritional goal, the size of fruits and seeds are increased from the wild plant, and represent a better resource with for example, a higher protein content in the case of bean seeds (Evans, 1993).

The domestication of plants results from mutation, hybridisation, selection and drift (van Raamsdonck, 1993). One of the most striking steps in the domestication process is to induce tetraploidy. Tetraploid plants have larger seeds (facilitating the shooting of seedlings through the seed covering), larger underground and aboveground organs (which facilitate care of the plants and help to improve yield), and higher content of certain chemical combinations (Kupzow, 1980). In the case of domestication of the genus *Phaseolus*, it has been shown that seeds from cultivated varieties contain higher levels of proteins and minerals (Baldi & Salami, 1973; Delgado, 1988). Thus the cultivated plants provide a higher quality resource for humans, and also for insects that feed upon these plants (pests and their associated parasitoids) (Benrey *et al.*, 1998).

a) Effects of plant domestication on herbivorous insects:

Plant domestication leads to the loss of chemical defence (Evans, 1993): cultivated varieties do not produce the same quality and quantity of chemicals than their wild counterparts. In general, domesticated plants have lower levels of chemical defensive compounds than wild plants (Rhoades, 1979; Evans, 1993; Rosenthal & Dirzo, 1997). This decrease of chemical compounds in the edible parts (fruit, seeds) can be explained as a consequence of selection for a better taste or a higher nutrient composition. In the case of

beans, it has been shown that wild bean seeds contain an α-amylase inhibitor protein that does not permit the development inside these seeds of the pest *Callosobruchus chinensis* or *C. maculatus* (Ishimoto & Katamura, 1989). But several cultivars (Ofuku 5 and Ofuku 6) do not contain any seed α-amylase inhibitor protein (Ishimoto *et al.*,1995). In the domestication of corn, wild plants resist better the attack by the phytophagous pest *Diatraea grandiosella* than modern cultivars (Rosenthal & Dirzo, 1997), showing a trade-off between the selection for growth and yield versus defence against herbivores. In the plant *Capsicum annuum*, some wild populations were found resistant to the geminivirus PHV, a pathogen for which none of the available commercial varieties had resistance (Hernandez-Verdugo *et al.*, 2001b). In this plant, the wild forms have a higher level of genetic variation than the cultivated forms, and are a valuable genetic resource which could be used in breeding programs (Hernandez-Verdugo *et al.*, 2001a).

Others chemical compounds can also be altered during the process of plant selection such as, volatiles that represent an indirect defence and are used as cues by the parasitic wasps during the host localization process (for review see Turlings & Benrey, 1998; Fritzsche-Hoballah & Turlings, 2001). In maize for example, a comparison of several maize cultivars and wild forms (Teosinte) showed a high genetic variability in odour emissions both in quantity and quality (Gouinguené *et al.*, 2001). The total amount of volatiles released after inducing mechanical damage to the plant, was significantly different among maize cultivars as well as among the teosintes. Moreover, the composition also varied, particularly for the caryophyllene, and the ratio among monoterpenes and sesquiterpenes. On *Phaseolus coccineus*, Aebi (1999) found no difference between the cultivated form, *P. c. coccineus*, and its wild form, *P. c. formosus*, in the total blend emission. But, when analysing each compound separately, wild *P.c. formosus* emitted significantly a higher amount of one compound ("D")

than the cultivated form. In other cases, such as for crucifer, domestication has not altered the production of volatile compounds used by insects for the host location (Benrey *et al.*,1998).

On citrus cultivars, which differ in suitability for the growth and survival of the California red scale, Hare & Luck (1991) found variability in the sex ratio of *Aphytis melinus*, an ectoparasitoid of the California red scale. Wasps from scales reared on *Citrus limon*, produced twice the proportion of females progeny than wasps from scales reared on *C. paradisi, C. sinensis* or *C. unshiu*. Idris & Grafius (1996) showed variation in the relationship and fitness parameters between the diamondback moth (*Plutella xylostella*) and its parasitic wasp (*Diadegma insulare*) according to their host plant variety, wild versus cultivated Brassicae. On cultivated host plants, the larval survival of *P. xylostella* was higher, and their development time is shorter than on wild forms. The parasitism rate by *D. insulare* was lower on the wild forms. The better performance of the herbivore and the parasitoid on cultivated plant was also demonstrated by Benrey *et al.,* (1998) on two biological systems: one involving the herbivore *Pieris rapae* and its parasitoid *Cotesia glomerata* on wild and cultivated *Brassicae*, and the other involving plants of the genus *Phaseolus*, the pest *Zabrotes subfasciatus* witch is parasitized by *Stenocorse bruchivora*.

b) Plant Selection : the case of Phaseolus sp.

One of the most important plants in Mexico for the use of seeds as a food resource of humans is the bean *Phaseolus sp*. Because wild beans are adapted to colonize disturbed environments, they may have been a favourable plant material for domestication (Delgado *et al.,* 1988). The beans were domesticated for their seeds which may be stored dry.

Evans (1976) reviews the four different cultivated species of *Phaseolus* in the New World, with an estimation of the number of years in domestication (Kaplan, 1965; Kaplan *et al.*, 1973):

- *P. vulgaris*: the common bean, or also called French bean (7700 B.C.),
- *P. coccineus*: the runner or scarlet runner bean (2000 B.C..),
- *P. acutifolius* var. *latifolius*: the Tepary bean (5000 B.C.),
- *P. lunatus*: the Lima or Madagascar bean (7700 B.C.).

The two main cultivated species of *Phaseolus* are *P. coccineus* and *P. vulgaris*. For both of them, there are several cultivated forms. Cultivated and wild seeds differ in phenology, morphology and nutritional quality.

Phaseolus vulgaris presents two seed types : some races with small seeds, domesticated since 7000 B.C., in Middle America, while other races have large seeds, domesticated since 7800 B.C. in South America. Also for *P. lunatus* exist races with small seeds from Middle America, domesticated since 1400 to 1800 B.C. and races with large seeds from South America since 4500 B.C. Thus, there are two separate centres of domestication for both species: one in Mexico which leads to races with small seeds, and a second one in Peru, derivated by migration from the first one, which leads to races with large seeds. But Heiser (1965) hypothesizes that domestication in Peru was an independent domestication from the closely related *P. arborigineus* Burkhart, which occurs wild in this area. This separation in the centres of domestication leading to the size variation was not demonstrated for *P. acutifolius* and *P. coccineus* (Evans, 1976).

Viable and fertile hybrids can be obtained between *P. vulgaris* and *P. coccineus* (Smartt, 1970; Wall & Wall, 1975). Crossed with *P. acutifolius*, both of these species produce viable but sterile hybrids. Miranda & Evans (1973) showed differences in the reciprocal crossability between cultivated forms of *P. vulgaris* and *P. coccineus*. Hybrids obtained from a female *P. vulgaris* crossed with a male *P. coccineus* produce easily fertile bean plants while the reciprocal is more difficult. This is not the case if wild species are used. This suggest that

the unilateral incompatibility has developed under cultivation which represents a first effect of the domestication process.

Smartt (1993) reviewed the main effects produced by the domestication on the genus *Phaseolus*, and specifically for *P. vulgaris*, *P. lunatus*, *P. coccineus* and *P. acutifolius*. These are:

- Gigantism, expressed in larger seeds, larger pods and leaves;
- Seed dispersal mechanisms, with the suppression of the normal dehiscence mechanism;
- Changed growth form: from a rampantly growing indeterminate wild plant to a dwarf determinate cultivar;
- Changed life-form: both annual and perennial forms are found in cultivated *Phaseolus* species and their wild relatives, but in *P.vulgaris* domesticated forms are typically annual;
- Loss of seed dormancy: wild plants of *Phaseolus* have usually a seed dormancy whereas it does not exist in cultivated varieties;
- Loss of photoperiodic sensitivity that allow the cultivation of beans in temperate zones.
- Biochemical changes such as a decrease in level of toxic compounds (i.e. hydrogen cyanide) in the cultivated forms.

The cultivated forms of beans have also lost the ability to produce some secondary compounds such as lectins (Sotelo *et al.*, 1995) or phenolic and cyanogenic compounds (Vanderborgth, 1979), and do not produce flavonoides found in the wild forms (Linding *et al.,* 1997). Seeds from the cultivated forms have higher concentration of proteins and minerals than the wild seeds (Delgado *et al.*, 1999).

Overall, the chemical composition, the larger seed size and the thickness of the seed coat of the cultivated seeds (Smartt, 1988; Delgado, 1988; Evans, 1993) are characteristics that will affect the interactions between the plant and the phytophagous hosts that feed on them (Guzman-Maldonado *et al.*, 1996; Callejas, 1996; Ceballos, 2002).

c) The case of Phaseolus vulgaris*:*

According to Smartt (1993) the evolutionary advance under domestication is considerably greater for *P. vulgaris* than for *P. lunatus*, *P. coccineus* and *P. acutifolius*. This species has the greatest pool of genetic variability.

The wild common bean (*P. vulgaris*) has several native names such as "frijol de mote, frijolillo, frijol de ratón, frijol de coyote" (review in Delgado *et al.*, 1988). The foliage is used to feed the livestock and wild grazing animals. Dry seeds are gathered to be used for human consumption in different Mexican states such as Guerrero, Morelos and Oaxaca.

From an analysis of 306 landraces of cultivated common bean in Latin America, and the use of electrophoresis of allozymes, Singh *et al.*, (1991a, 1991b) confirmed the existence of two major groups, each one subdivided in subgroups: a Mesoamerican group (with five subgroups) and an Andean group (with four subgroups) , with distinctive morphology, adaptations and resistance for disease. In another study, they divided these groups in races: Durango, Jalisco and Mesoameric races for the Middle America group, and the races of Chile, Nueva Granada and Peru for the Andean South America group (Singh *et al.*, 1991c). This group separation has been confirmed with DNA fingerprinting analysis (Sonnante *et al.*, 1994). However, the crosses between these two groups do not always produce viable hybrids. There is an hybrid weakness due to the genes Dl1 and Dl2, present in wild *P. vulgaris* and introduced among cultivars presumably through domestication (Koinange & Gepts, 1992).

Some results also provided indications of gene flow from wild to cultivated beans (Singh *et al.*,1991a; Gepts, personal communication).

Recently, Debouck *et al.,* (1993) identified wild common bean populations in Ecuador and northern Peru, where they had never been described before. They conducted an analysis of the distribution of the wild *P. vulgaris* and found an ecological distribution in relatively dry environments with intermediate temperatures, known as "dry mountain forest".

In Latin America and some parts of Africa, cultivars of *P. vulgaris* are the source of the main part of protein food of the inhabitants, being grown mainly for the dried pulse. In Europe and the United States they are grown mainly for the green immature pods which are eaten as a vegetable.

Usually a mixture of cultivars is sown, and in many places the crop is interplanted with others crops species such as maize, cotton, sweet potatoes or coffee (Pursglove, 1968). The common bean is of little importance in India and most tropical Asia, where indigenous pulses are preferred (Purseglove, 1968).

In the 16th century, Spaniards and Portuguese introduced the common bean in Europe, and it reached England in 1594. Then it was also introduced to Africa and other countries of the Old World. *Phaseolus vulgaris* is now widely cultivated in many parts of the tropics and subtropics and throughout the temperate regions. The habitat of wild beans may have actually been extended as humans accentuated disturbances trough agricultural settlements (Delgado *et al.*, 1988; Debouck *et al.,* 1993).

Modifications in *P. vulgaris* induced by domestication :

Koinange *et al.,*(1996) evaluated the genetic control of the "domestication syndrome" ("morphological and physiological traits that distinguish wild progenitors and cultivated

descendants") and found three genomic regions. The first one affects the growth habit and phenology, the second one affects the seed dispersal and dormancy, and the last one affects the fruit and seed size. All of them are the main traits that determine adaptation to a cultivated environment. Nakamura (1986) observed that wild plants had smaller pods and smaller seeds than cultivated forms, but they had a higher number of flowers, a higher number of mature pods and as a result, a higher number of mature seeds.

The dry seeds are rich in protein (about 22 %). The other components in the seed are on average: carbohydrate (57.8 %), fibre (4 %), fat (1.6 %), ash (3.6 %), and water (11 %). The green immature pods (snap bean) contain about 22 % of protein, 0.2 % of fat, 6.3 % of carbohydrate, 1.4 % of fibre, 0.8 % of ash and 85.2 % of water (Purseglove, 1968). Baldi and Salami (1973) found that the protein content of the seed of *Phaseolus vulgaris* var. *mexicanus* is of the order of about 35 %, higher than *P. vulgaris* var. *vulgaris* and than the other cultivated *Phaseolus* with also higher arginine levels (8,05 versus 5.91 %).

An obvious effect of domestication in *P. vulgaris* is the modification of the growth habit. While the wild bean forms respond to short days due to the photoperiod sensitivity, some cultivars of temperate countries have been selected for day-neutrality or tolerance of long days.

In comparison with the wild forms which grow between 16 to 22 ° C, most of the cultivated varieties of *P. vulgaris* grow in areas in which the mean temperature during the growing season ranges between 17.5 and 25 °C (Laing *et al.*, 1984). Studies on cultivars showed that selection on heat tolerance can be done by genetic crosses between the cultivars (Shonnard & Gepts, 1994).

There is also a reduction in the number of branches, of leaves and an increase in leaf size and stem diameter in cultivated plants. Seed number per pod has changed, where up to 9 seeds are found in wild forms but rarely more than 5 in most cultivars. The pods length

increased from 6 to 30 cm, and from 100 to 1000 mg for individual seed weight. There is a reduction of the dehiscence and in fibre content in the cultivated forms. The changes in the pod structure and dehiscence are determined according to the bean variety will be used for dry seed production or green pod production (Evans1976).

Moreover there is an increase in permeability of the seeds to water which is an important property for uniform germination and for easiness of cooking. Due to their hard seed coat, the wild seeds need a longer cooking time than cultivated varieties: 6 hrs at 100°C (Miranda, 1974). In many tropical countries, bean are usually consumed as dry bean whereas in temperate countries varieties have also been developed for fresh pod consumption and for processing as frozen vegetable (Evans, 1976).

Crosses between wild and cultivated beans are easily done and yield viable and fertile plants (Evans, 1980). Intermediate forms, the so-called "weedy" types, result from outcrosses between wild and cultivated types. They can also represent escapes from cultivation or may be a product of gene flow between varieties, by means of insect pollinators such as bumblebees attracted by the flowers, even if *P. vulgaris* is mainly a self-fertilized plant (Smartt, 1993). They have a lower number of seeds per pod (4.2 versus 5.8) and larger seeds (11.9 g versus 6.3 g/100 seeds) than the wild plants. They have also a different colour as cream, pink or yellow (Vanderborght, 1983) compared to the dark and mottled colours of the coat of wild forms which may camouflage the seeds against the soil background, and avoid predation which is not the case with the light colour of the weedy forms (Smartt, 1993).

The domestication process also induced a decrease in the variability of phaseolin. Phaseolin is a major storage protein in *P. vulgaris*, and it exhibits higher levels of variability among wild common beans than in cultivars (Gepts *et al.*, 1986).

Some of the compounds of the seeds protect them against phytophagous insects, such as the α-amylase inhibitor protein which inhibits the larval midgut α-amylase activities of *Callosobruchus chinensis* and *C. maculatus*. Larvae of these bruchids die before the second larval instar when fed on seeds containing this inhibitor (Ishimoto & Katamura, 1989). The inhibitor activity is linked with the presence of arcelin variants. But in *P. vulgaris* cultivars, *Zabrotes subfasciatus* and *Acanthoscelides obtectus* are tolerant to this inhibitor. Ishimoto *et al.,* (1995), identified a novel inhibitory activity in the α-amylase inhibitor (αAI-3) against *Z. subfasciatus* in seeds of wild *P. vulgaris* mainly distributed in Mexico. Wild *P. vulgaris* accessions exhibited a larger diversity of seed α-amylase inhibitors. In contrast, only four α-amylase inhibitor types were detected in cultivars accessions. Several cultivars such as, "Ofuku 5" and "Ofuku 6" do not contain any seed α-amylase inhibitor proteins (Ishimoto & Katamura, 1991). This confirms the study of Van Schoonhoven *et al.,* (1983). These authors had reported high levels of resistance to both *A. obtectus* and *Z. subfasciatus* in wild common beans from Mexico, whereas a large number of cultivated beans had exhibited low or no resistance to these two bruchids (Van Schoonhoven and Cardona, 1982). The resistance to *Z. subfasciatus* in wild *P. vulgaris* is clearly and closely linked to the presence of the arcelin-1 and arcelin-5 locus (Paes *et al.*, 2000; Gerhardt *et al.*, 2000) even if arcelin-5 proteins alone are not sufficient to achieve adequate resistance against *Z. subfasciatus* (Goossens *et al.*, 2000). These proteins have growth inhibitory effects for the larvae, by an alteration of the gut structure an its penetration into the hemolymph. At present, studies are being conducted on breeding programmes aimed at introgressing high levels of resistance to *Z. subfasciatus* in *P. vulgaris* cultivars (Goossens *et al.*, 2000). In some cases, genetic modifications in the seed protein composition (Arcelin versus Phaseolin) on SMARC lines, leads to a highest level of resistance to the bruchids *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Hartweck *et al.,* 1997).

In response to such variations on the bean plant, it is evident that insects that feed on it must have to adapt to these changes. Than, the present study focused on the effect of the variability in the seed quality on the development and performance of one phytophagous insect and its parasitic wasps.

III. Biological system:

The studied tritrophic system includes a Leguminosae as the host plant (*Phaseolus vulgaris* L.), a bruchid beetle (*Zabrotes subfasciatus*) as the phytophagous host, and two parasitic wasps (*Stenocorse bruchivora* and *Dinarmus basalis*).

a) Phaseolus vulgaris:

A recent study on the phylogenetic analysis of *Phaseolus* by used of DNA, revels 9 monophyletic species clades (Table 1) (Delgado *et al*., 1999; Delgado 2000).

P. vulgaris	*P. vulgaris*, *P. coccineus*, *P. albescences, P. costaricensis, P. polyanthus*, *P. acutifolius.*
P. filiformis	*P. angustissimus, P. filiformis.*
P. lunatus	*P. lunatus, P. mollis, P. pachyrhizoides, P. augustii, P. bolivianus, P. viridis, P. lignosis.*
P. polystachios	*P. salicifolius, P. smilacifolius, P. polystachios, P. sinuatus, P. maculatus, P. jaliscanus, P. sonorensis, P. ritensis, P. juquilensis*, *P. marechalii*, *P. xolocotzii.*
P. leptostachycus	*P. leptostachycus, P. micranthus*, *P.macvaughii.*
P. pauciflorus	*P. pauciflorus, P. perpexplus, P. parvelus, P. plagiocylix, P. pluriflorus, P. tenellus, P. nelsonii, P. amblyosepalus.*
P. tuerckheimii	*P. tuerckheimii, P. oligospermus, P. macrolepis, P. chiapasanus*, *P. hintonii*, *P. zimapanensis*, *P. xanthotrichus.*
P. pedicellatus	*P. pedicellatus, P. neglectus, P. grayanus, P. oaxacanus, P. glabellus.*
P. microcarpus	*P. microcarpus.*

Table 1: Phylogenetic analysis of *Phaseolus* (Delgado *et al.*, 1999; Delgado, 2000)

In 1997, Graham and Ranalli reviewed the origins, the general botany and the different cropping systems used for bean production of *P. vulgaris*. The centre of origin of *P. vulgaris*

is located in the Mexican mountains, where the greatest diversity of wild and cultivated forms is concentrated. *P. vulgaris L*. is distributed both in Middle America (Mexico and Central America) and in the Andes of South America. The plants grow from 500 to 1900 m, but mostly between 1500 to 1900m (Delgado *et al.* 1988). *P. vulgaris* var. *mexicanus* (Photo 1) grows in a climate ranging from semi-hot or temperate subhumid to hot semidry, all with rain in the summer (from may to October), with temperatures between 16 to 22 °C and annual rainfall ranges from 550 to 1000 mm. The growing period extends from May to November, after which the plants mature coinciding with the return of the dry season (Delgado *et al.* 1988).

The wild relatives of crop plants constitute the raw material from which present day cultivars have been derived (Delgado *et al.* 1988). *Phaseolus vulgaris* has been cultivated for 7000 years in Middle America and for 7680 B.P. in Peru (Kaplan, 1965, Kaplan *et al.*, 1973). It is believed that one of the domestication regions could be the state of Jalisco (Kaplan, 1965; Gepts *et al.*, 1986). Now it is the best known and the most widely cultivated species of *Phaseolus*. This plant is widely used by human populations and its domestication has been extensive (Photo 2).

Brief description of *P. vulgaris* var. *mexicanus* (Fig. 1):

The following description is based on Delgado *et al*. (1988): it is an annual plant or rarely, a short-lived perennial, developing from elongate or fibrous root system. The flowers are small, 13-18 mm long, normally pink, pale purple or white in colour. The pods are straight to slightly falcate, up to 62.9 mm long , and 5.5 mm wide and weigh approximately 0.46 g. Seeds are oblong, reniform or trapezoidal in shape, 3.5 to 11 mm long, and 2.5 to 5.5 mm wide. The testa may be greenish, beige, yellow, pale or dark brown, grey, black, or pinto usually striped or mottled with black. There is an opening on the pod suture that permits entrance of pests such as bruchid beetles but also of parasitoids.

It is important to note that crosses between wild and cultivated forms are frequent, and sometimes we can note occasional hybridisation with wild *Phaseolus coccineus* and subsequent backcrossing of the hybrids (Wall & Wall, 1975; personal observation).

Delgado (1985) separated the varieties of wild *P. vulgaris* according to their localization. The Mesoamerican *Phaseolus* form (Mexico and Central America, in tropical deciduous to mesophytic forest) is *P. vulgaris* var. *mexicanus*, whereas the South American form is called *P. vulgaris* var. *arborigineus* (Burkart) Baudet, and it is located in the mesophytic forests on the eastern side of the Cordillera Andina. A complete review of the differences found between these varieties is found in Delgado *et al.* (1988). The main differences are the number of seeds per pod (less in the *aborigineus* variety, 5 to 8 versus 8 to 10), and the seed weight (100 seeds of the *arborigineus* variety are heavier 11.6 versus 3.5 g). The seeds of *P. vulgaris* var. *arborigineus* showed a faster but more irregular germination than seeds of the mexicanus form which are hard and impermeable to water.

All stages and plant structures of *P. vulgaris* are attacked by a variety of natural enemies (patogens, herbivores and seed predators). Zaumeyer & Thomas (1857) and Miranda (1967) review some pathogens associated with the wild common beans in the Middle America. But with the introduction of beans in the Old World, these diseases are worldwide. For example, in the fungi group, we find the Anthracnose (*Colletotrichum* spp.) which is one of the most destructive disease of *P. vulgaris* , appearing on the stems and leaf veins in the guise of small angular brick-red lesions, and now almost worldwide, the root rots (*Rhizoctonia* sp., *Fusarium* spp.) also found in England and Australia, and the white mold (*Sclerotinia* spp.). There are also bacteria (*Xanthomonas spp., Pseudomonas spp.*) and mosaic viruses transmitted by aphids and infected seeds (Purseglove, 1968). The virus causes a ruffling and yellow mottling leaves. These authors also list some pests such as, the leaf miner (*Liriomyza* spp., *Chalepus* spp.), spider mites (*Paratetranichus* spp.), white flies

(*Trialeuroides* spp.), leafhoppers (*Empoasca* spp.) and of course seed weevils (Bruchidae) which are the most serious bean pest of dry seeds (*Acanthoscelides* spp. and *Zabrotes subfasciatus*).

b) Zabrotes subfasciatus:

Previously named *Spermophagus subfasciatus* (Boheman, 1883) (Coleoptera: Bruchidae : Amblycerinae), it is one of the main pests of field crops and stored beans (Leroi *et al.* 1990; Ramirez, 1991) in Mexico and Central America where its called "gorgojo mexicano del frijol". This bruchid (Photo 3) attacks mainly *Phaseolus* plant species, but also other plants in the family Leguminosae such as, *Vicia faba* (Sànchez, 1992), *Vigna sinensis* and *Vigna subterranea* (Hill, 1990) (see Romero & Johnson, 1999, for a review on the host plant species). It lives around 28 days at 70 % r.h. and 27 °C (Rios Casanova, 1998). Optimal conditions for its development are 32°C with 70% r.h., but this beetle can survive with a minimum of 20°C to a maximum of 38°C (Hill, 1990). Females enter in the mature pods of *Phaseolus* through an opening ("dehiscence slit") in the pod suture close to the pedicel. They lay an average of 40 to 50 eggs in their life, which they glue on the seed coat. The maximum fecundity of females is around 55 eggs (Dendy & Credland, 1991). Incubation time of the egg is around 6 days. Then first instar larvae burrow into the bean seed where they complete their development (4 larval instars), pupate, and emerge as adults. Development time is around 26 to 28 days inside the seed, but it is very dependant on the temperature. At 27°C, with 70 % h.r. around 80% of adults emerge after 34 days (Dendy & Credland, 1991). From the last experiment, it was estimated that 75% of the larvae inside the seed produce adults at low or moderate density. Beetles require neither food nor water after their emergence.

Pimbert & Jarry (1988) studied the pattern of oviposition of *Zabrotes subfasciatus* on two bean species *Phaseolus lunatus* (wild form) and *P. vulgaris* (cultivated form) in relation with the pod morphology. On *P. lunatus*, females tends to lay their eggs uniformly among

seeds inside the pods of *P. lunatus*, whereas inside the pods of *P. vulgaris*, the egg distribution patterns are random or aggregative.

This bruchid presents a strong sexual dimorphism. (Ramirez, 1991; Callejas, 1996). Female size is around 2.47 mm, black with white stains on the outer wings. Males are smaller, coffee-colored without the white stains on the outer wings. There is a strong correlation between the weigh of females at emergence and their fecundity (Dendy & Credland, 1991; Callejas, 1996).

c) Stenocorse bruchivora:

Previously named *Glyptocolastes bruchivorus,* Crawford, 1909) (Hymenoptera: Braconidae) *S. bruchivora* is a solitary ectoparasitoid of bruchids. It is an idiobiont synovigenic wasp endemic of America (Photo 4). The fact that only parasitizes bruchids makes it a specialist. In Mexico, it's main hosts are *Zabrotes subfasciatus* (Coleoptera : Bruchidae), *Acanthoscelides obtectus* (Say) and *A. obvelatus* (Bridwell) (Coleoptera: Bruchidae) on bean plants (*Phaseolus* spp.) (Perez & Bonnet, 1984). Hetz and Johnson (1988) present a list of others potential bruchid hosts for this wasp such as *Cariedes spp., Megacerus spp., Merobruchus spp., Sennius spp., Stator spp.,* on several host plant species such as *Acacia spp., Calopogonium spp., Cassia spp., Mimosa spp.,* and in different countries (USA, Mexico, Guatemala, Honduras, Panama).

Females and males have a length between 2.5 to 4.5 mm (Crawford, 1909). The strong sexual dimorphism is the external ovipositor at the end of the female abdomen. In this species, male are polygamic whereas female previously mated are not receptive to the male courtship (Perez & Bonnet, 1984).

The females of *Stenocorse bruchivora* paralyse totally and definitely the larval host inside the bean before laying a single egg on its surface. They parasitize the third and fourth

instars and occasionally pupae (Perez & Bonnet, 1984; Rios, 1997). Reyes (1999) found a better development on *Zabrotes* larvae of 17 and 19 days old, than 14 or 21 days old on *P. vulgaris* seeds. These host ages induce a better parasitism rate (15.5 and 10.8 % versus 6.37 and 2.76) and produce parasitoid wasps of a larger size (tibia size: 1.6 mm versus 1.2 mm) and with greater longevity. On these hosts the sex-ratio was also biased toward females.

Perez and Bonnet (1984) indicate a development time of 22 days for the males and 24 days for the females under 24°C with 70 % r.h. For Reyes (1999) the development time is influenced by the host larval stage and is around 18.4 days for the males, and around 21 days for the females, on 17 days-old host larvae, in the same environmental conditions than Perez and Bonet (1984). As for other parasitoid species (Podoler & Mendel, 1979), males emerge from 24 to 48 hours before females. Another factor that affects the development time of *S. bruchivora* is the quality of the host. On *Phaseolus coccineus coccineus,* parasitoids that emerge from higher quality hosts develop faster than parasitoids that emerge from poor quality hosts. The development time is shorter in relation with a greater host quality (Rios, 1998). As for other species (Syme, 1977; Roff, 1992; Stearns, 1992; Visser, 1994), adult longevity of *Stenocorse* is positively correlated with the size of the individual which in turn is influenced by the size of the host (Reyes, 1999). Longevity is also influenced by the feeding history of the adult wasp: if the wasp can feed with honey or water and sugar, its life cycle is longer (between 11 to 15 time more). For example, without a bruchid host to parasitize, a *S. bruchivora* female lives 7.4 days (+/- 0.53) without food, or 107,4 days (+/- 6.34) with food supply. Males have a shorter longevity, between 56 to 58.6 days if they have food. Female have a reduced longevity if they parasitize host (less than half of a naïve female) (Reyes, 1999). In their laboratory study, Perez & Bonet (1984) estimated 67 days of mean average longevity. The size of the wasp is also positively correlated with its fecundity with ($p = 0.0001$) or without ($p = 0.0009$) food supply. Bigger females lay more egg (Reyes, 1999).

The same study showed that females of *Stenocorse* do not require food at the adult stage to be able to parasitize the host, which is not the case with other the parasitoid species (Vinson & Barbosa, 1987). However, their fecundity is 16 time more if the females receive food. On *Acanthoscelides obtectus* as a host, a single *Stenocorse bruchivora* female is able to lay 43 eggs during her lifetime (Perez & Bonet, 1984).

***d) Dinarmus basalis*:**

Previously named *Entedon basalis*, (Rondani, 1877) (Hymenoptera: Pteromalidae) (For other previous names, see the review by Rasplus, 1989), it is an idiobiont solitary ectoparasitoid on larvae and pupae of several bruchid species developing in Leguminosae seeds (Verma, 1991) (Photo 5). This species is originated from Africa (South Africa, Niger, Cameroon, etc..), but due to seed trade it colonized America (U.S.A, Mexico, Peru), Asia (Bangladesh, India, Pakistan) and sometimes it has been found in Great Britain and France as the result of seed importation. Relative to *Stenocorse bruchivora*, *D. basalis* is considered a generalist since it is able to parasitize a wider range of hosts, in a wider range of plants such as *Vigna subterranea*, *V. unguiculata*, *Rhynchosia buettneri* (Rasplus, 1989). Its main hosts are *Callosobruchus maculatus*, *C. subinnotatus*, *Specularius erythraeus* and *Bruchidius atrolineatus* en Africa, *Callosobruchus chinensis* and *C. maculatus* in Asia, *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in America (Rasplus, 1989).

Length is around 2.3 to 2.9 mm for the female, and between 1.7 to 2.6 mm for the male. Adults emerge from 13 to 14 days after egg-laying. Males have a shorter development time, tending to emerge one day before females (with 33° : 23° C, L12:D12: Gautier *et al.*, 1997). Islam (1991) found a development time of 284 h (11 days) for the males versus 308.14 h (13 days) for the females. Males present a white yellowish spot on the gaster.

D. basalis has proved to be a very good biological control agent against bruchids. The introduction of *D. basalis* in populations of *C. maculatus*, *C. chinensis* or *Bruchidius*

atrolineatus, cause a major reduction or a total suppression of bruchid numbers but the success is very dependent on the prevailing conditions of storage in the region (Ouedraogo *et al.*, 1996; Islam & Kabir, 1995; Sanon *et al.*, 1998). *Dinarmus basalis* is synovigenic: females produce eggs throughout their life. A female is able to lay about 15 eggs in 24 h under the most suitable conditions (high density of large hosts, minimum search time) (Nishimura, 1993). It utilizes the oviposition – marking pheromone of the host weevils as a host-recognizing kairomone (Kumazaki *et al.*, 2000). Mated females lay more diploid eggs on larger hosts while smaller hosts are parasitized with haploid eggs (Nishimura, 1993, 1997). Virgin females live more than mated females (Nishimura, 1997). If they do not parasitize hosts, virgin and mated females have the same longevity. This phenomenon is due to the fact that mated females oviposit fertile (diploid) eggs and virgin females only haploid eggs which need less energy to be produced. In this case virgin female choose the smaller hosts to oviposit. In addition virgin females produce more eggs than inseminated ones (Nishimura, 1997).

Before they deposit an egg, females paralyze the host and feed by partially sucking out body fluid from the host. Females prefer to oviposit on 4th instar larvae and prepupae. These instars induce a shorter development time, a bigger size and a sex-ratio biased toward females (Islam, 1994). Females tend to avoid conspecific superparasitism and discriminate previously parasitized hosts (Gautier *et al*., 1996). The information on a previously parasitized host is obtained by deterrent markers produced by the first parasitoid egg (Gauthier & Monge 1999). If the hosts are previously parasitized, the offspring sex-ratio is male biased (Gautier *et al*., 1997). The proportion of males increases as the number of females parasitizing the same host patch increases. It has been shown that females can manipulate the egg fertilization during the oviposition phase (Gautier *et al*., 1997).

This species has also been well studied for its pre and post-emergence learning ability (Monge & Cortesero, 1996). This study showed that the response to host cues is determined by a pre-emergence learning process which takes place when females are in contact with the seed and the host larval remains. Moreover, an associative learning effect takes place when a female parasitizes the host.

Competition between this parasitoid with other species (*Stenocorse bruchivora* and *Eupelmus vuilleti*) has also been extensively studied and will be presented and discussed in the next chapter.

e) The localities and populations :

Three populations were selected for this study, all of them located around Mexico City (Map 1), and all of them are associated with *Zabrotes subfasciatus*, *Stenocorse bruchivora* and *Dinarmus basalis.* These populations are well known and used for previous experiments (Leroi et al, 1990; Callejas, 1996, 2002). They are: Atila (18°37 N, 98°34 W) in the state of Puebla, Malinalco (18°57 N, 99°30 W) state of Mexico and Tepoztlan (19°00 N, 99°07 W) state of Morelos. The name of these populations corresponds to the nearest known point. Atila is the name of the river crossing this locality. Malinalco is situated within an archaeological site with the same name and Tepoztlan is located along a road that leads to the village of the same name.

One important characteristic of these 3 populations is that they are geographically isolated from each other. They are separated by approximately 30 to 80 km**,** and located at different altitude range which lead to a different composition vegetation (Table 2).

This geographical range leads to phenological differences in the flowering and maturation of pods and seeds of *Phaseolus vulgaris* (Callejas, 2002). During the field season 2000 / 2001, the period of collection of seeds in Atila was closed to the last week of January, while mature

pods in Malinalco were found until mid February. In Tepoztlan, mature pods were available from the end of January until the middle of March, with emergence in the laboratory of the last wild *Stenocorse* parasitoids the first week of May 2001.

Population name	**State** (latitude and longitude)	**Altitude (m)**	**Phaseolus species**	**Vegetation**	**Climate**
Atila	Puebla (18°37N, 98°34 W)	1250	PVS (PLS)	Secondary vegetation in timbered drift (*Taxodium sp.*)	Aw°(w)
Malinalco	Mexico (18°57N, 99°30W)	1880-1900	PVS (PCS)	Forest of tropical deciduous trees (secondary vegetation) and oak forest	$A(C)W^{2}(w)big$
Tepoztlan	Morelos (19°00N, 99°07W)	1900	PVS PCS	Oak forest	$A(C)w^{1}(w)$

Table 2: general information on the 3 bean localities used in this study (from Leroi *et al.*, 1990). PVS: *Phaseolus vulgaris var. mexicanus*; PCS: *Phaseolus coccineus formosus*; PLS: *Phaseolus lunatus var. silvestre*. The climate is done according to Köppen modified by Garcia (1973).

These populations also differ in size. Atila is the smaller one with less than three hectares where bean plants are scattered. Malinalco is the most important one, with at least 6 hectares. The Tepoztlan population has an intermediate area of 4 hectares.

There are also differences in the rate of infestation by bruchid beetles. From 1997 to 1999, the infestation rate of seeds by bruchids was the most important in Malinalco, and the smaller rate of infestation was found in Atila (Callejas, 2002). But as for all natural system, these values are likely to change from one year to the next. In Atila, the infestation rate doubled during the 1998 /1999 field season (Callejas, 2002). They also vary in composition of other *Phaseolus* species and varieties. For example, Atila is located in an agricultural region were we can find both wild and cultivated bean varieties among sugar cane fields.

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Photos:

Photo 1: Wild pods of *Phaseolus vulgaris* (Mexico) (E. Campan)

Photo 2: Seeds of *Phaseolus vulgaris*; a: wild form; b,c,d : cultivated forms. b: Black bean; c: Red Kidney; d: Yellow bean.(E. Campan).

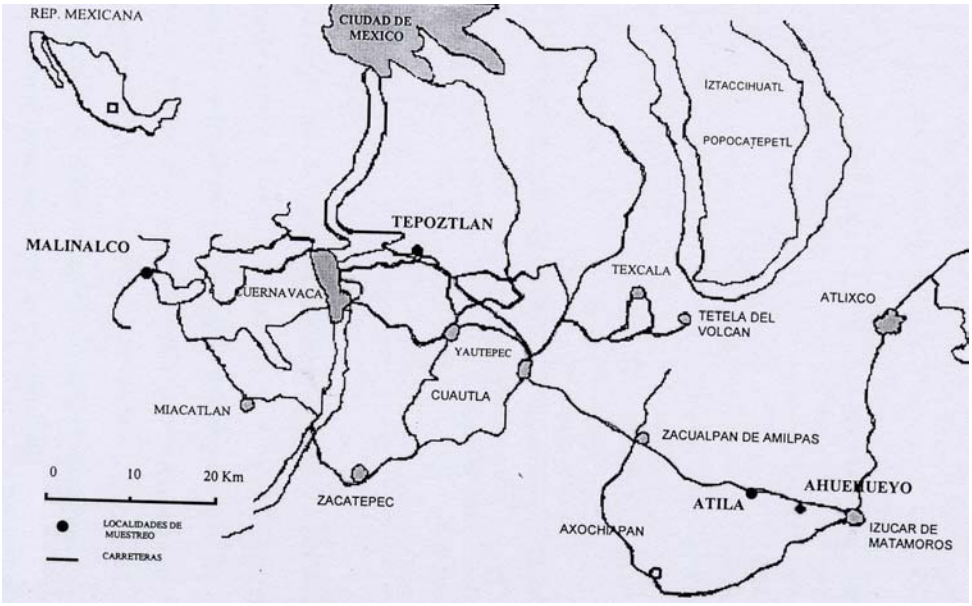
a b

c d

Photo 3: Female *Zabrotes subfasciatus* on seeds of *Vigna unguiculata.* (Y. Borcard)

Photo 4: Female *Stenocorse bruchivora* on wild seed of *P. vulgaris* (Y. Borcard)

Photo 5: Female *Dinarmus basalis* on *V. unguicularis* (Y. Borcard)



Map 1: Localities were the wild seeds of *P. vulgaris* were collected ; (Anonym, 1981)

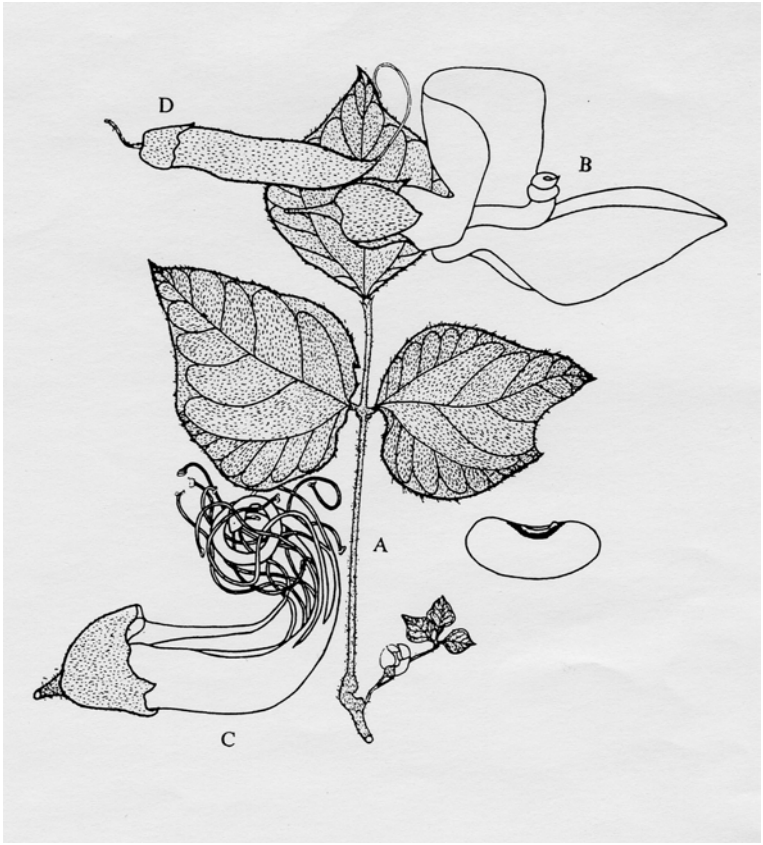


Fig. 1: *Phaseolus vulgaris*: the common bean. A: leaf; B: flower; C: flower with corolla removed; D: young pod; E: seed. (Purseglove, 1968).

THESIS OUTLINE :

Chapter 1: Environmental and genetic components for host use in *Zabrotes subfasciatus*.

Because the development of the phytophagous insect depends on the host plant, I investigated with a transplant experiment the role of the host plant quality on the bruchid beetles *Z. subfasciatus* reared for several generations on wild or cultivated seeds of *Phaseolus vulgaris*. A second part of this chapter involved the genetic component of phenotypic variability of the main fitness parameters such as, the fecundity, the sex ratio, the larval development time and the size of the phytophagous host *Z. subfasciatus* by comparing full-sib families established from a laboratory colony.

Chapter 2: Behaviour and performance of two bruchid parasitoids on wild and cultivated beans : specialist vs generalist.

In this chapter, I investigated the parasitism behaviour of two species of parasitic wasp that attack larvae of *Z. subfasciatus* in relation to the host plant quality. I investigated if the behaviour of the females and the fitness of the offspring, are affected by the seed type where the host was offered. To do this, I used wild and cultivated seeds of *Phaseolus vulgaris* and I compared two species that differ in their host specificity *Stenocorse bruchivora* (Hymenoptera: Braconidae), a specialist parasitic wasp endemic of America, and *Dinarmus basalis* (Hymenoptera: Pteromalidae), a generalist parasitoid originated from Africa but at present, spread throughout America and Europe.

Chapter 3: Inter-population variation in host use and performance in a bruchid parasitoid.

When a species inhabit different localities there is the potential for local adaptation, in which individual fitness will be higher in the original habitat than on novel habitat. This chapter investigated the presence of local adaptation in *S. bruchivora*. Through transplant experiment, I compared the behaviour and performance of individuals from three populations of *S. bruchivora* that attack bruchids on wild seeds of *P. vulgaris*.

Chapter 1

Environmental and genetic components for host use in *Zabrotes subfasciatus*

Abstract:

Phytophagous insects are dependent of their host plant for their successful development. In the special case of bruchid beetles, the seed is the structure on which they will complete their larval development. Due to the development of agriculture, some plants species have been domesticated for their use as a food resource. In the case of domestication of the genus *Phaseolus*, it has been shown that seeds from cultivated varieties contain higher levels of proteins and minerals and provide a higher quality resource for insects that feed upon these plants (the phytophagous pests and their associated parasitoids). In this study, I investigated the effects of bean domestication on *Zabrotes subfasciatus*, one of the main pests of field crop and stored beans in Mexico and Central America. Through experimental manipulations and quantitative genetics experiments, I determined the importance of environmental and genetic variation in the performance of this bruchid.

The results showed that the quality of the host plant mediated the performance and the oviposition behaviour of female beetles. Overall, performance of the bruchids was higher on cultivated seeds than on wild seeds. It was also found that whether a female originated from wild or from cultivated seeds affected her behaviour and the performance of her offspring. Females that originated from cultivated seeds were less willing to lay eggs on a novel host seed than females that originated from wild seeds. Thus, the performance and behaviour of *Z. subfasciatus* are not only affected by environmental factors such as, the quality of the seeds on which they develop, but also have a genetic basis.

Keywords: host plant, *Zabrotes subfasciatus*, full-sib families, egg quality.

Introduction:

Bruchid beetles are pests that feed on seeds of several crop plants. They develop as larvae within the host seed from which they will obtain all the resource required for their growth and development. Indeed, the larval offspring are directly dependent for its survival and its successful development on the mother choice. Thus, the oviposition decision of a bruchid female is expected to minimize the effects of larval competition and to maximize her life time fitness.

Several factors can influence the oviposition behaviour and the development of the bruchid:

- The host plant, and particularly the pod's and the seed's characteristics (size, coat, colour, chemical compounds). Even if the female lays eggs on some plant species, the seeds are not necessary suitable for their development. For example, in a no choice experiment, females of *Bruchidus atrolineatus* laid the same number of eggs on the host plant species (*Vigna unguiculata*) than on host plants that did not allow the development of the new generation such as, *V. subterranea* and *Phaseolus vulgaris* (Ofuya & Credland, 1996). Bruchids emerging from several varieties of the same host species can show differential performance on them. For instance, the variety of cowpea where larvae grew, modified the developmental period, the survival, the adult weight and the life-time fecundity of *Bruchidus atrolineatus*, whereas *Callosobruchus maculatus* did not succeed in their development (Ofuya & Credland, 1995; Ofuya & Awelewa, 1993). In the case of *Zabrotes subfasciatus*, fitness parameters are different depending on the experimental line of the bean host plant used (Ramirez - Tenorio, 1991). Pimbert & Jarry (1988) studied the pattern of oviposition of *Z. subfasciatus* on two bean species, *Phaseolus lunatus* (wild form) and *P. vulgaris* (cultivated form), in relation with the pod morphology, and demonstrated that on *P. lunatus*, females tend to lay their eggs uniformly among seeds inside the pods of *P. lunatus*, whereas inside the pods of *P. vulgaris*, the egg distribution patterns are random or aggregative. This was estimated using a dispersion index: its value tends towards a minimum when the egg distribution is uniform, and towards a maximum when all the eggs are concentrated on a single seed. Callejas (1996) found that females of *Zabrotes subfasciatus* laid more eggs on larger seeds of the cultivated subspecies of *Phaseolus coccineus* than on smaller seeds of the wild subspecies. This behaviour was confirmed using small and large wild seeds of *P. vulgaris* (Ceballos, 2002). In contrast, Plaza (2001) found that females prefer to lay eggs on smaller artificial

hosts (gelatine capsules) than on large capsules. Not only the number of eggs, but the size of these eggs can also be influenced by the host plant. Females of *Stator limbatus* lay large eggs *on Cercidium floridum* seeds (low quality host plant) and small eggs on *Acacia greggii* (high quality host plant) (Fox & Mousseau, 1996). This is an adaptively plastic character that allows them to readjust the egg size when they switch between these host plants (Fox *et al*., 1997). Bhattacharya & Banerjee (2001) found that the shape, the colour, the size, the texture and the thickness of the seed-coat of several hosts affects the egg-laying of females of *Callosobruchus chinensis*. Also the penetration of the larva into the seed varies and this has been shown to be inversely correlated with the phenol concentration of the seed. Guzman Maldonado *et al.* (1996) tested 17 cultivars for their susceptibility as hosts for *Z. subfasciatus* and *A. obtectus*. Among these, they did not find any significant differences in relation to the physical traits of the seeds. In the field, the dark seeds of *Phaseolus vulgaris* show a greater infestation rate by eggs of *Z. subfasciatus* than light ones, but this observation has not been confirmed with laboratory experiments (Ceballos, 2002). The same study showed that females oviposited more on large seeds, and changed the egg distribution pattern (random or regular) according to the number of the seeds in the pods, their size, and their colour. The number of available seeds appears to play a role in the oviposition behaviour of the females (Callejas, 1996). The females preferred a larger number of seed. Physico-chemical characteristics of the seeds of *Vigna unguiculata* have an influence on the number of eggs laid and the development of *Callosobruchus maculatus*. A comparative study on 9 lines of cowpea showed that the seed height explained 69.5 % of the variance in the number of eggs laid (Oigiangbe & Onigbinde, 1996).

The composition of the seed also influences the performance of bruchid beetles. The presence of tannic acids in some cowpea varieties significantly reduced the F1 progeny by reducing larval penetration, larval growth and development (Oigiangbe & Onigbinde, 1996). Among 17 cultivars of *P. vulgaris*, different lectin concentration influenced both the oviposition and the adult emergence of *Z. subfasciatus* (Guzman Maldonado et al.,1996).

- The bruchid genotype and its physiology. Genetic variability has been often estimated between and within bruchid populations. Even in the laboratory, were the conditions are standardized, variability in life history traits of bruchid beetles exists. *Callosobruchus maculatus* (Coleoptera: Bruchidae) has been well studied in relation to its life history parameters. The main results have shown that there is a strong relationship between maternal age, egg size and mating frequency. Maternal age influences the egg size (Fox, 1993a) and the offspring's performance (Fox & Dingle 1994). Egg size decreases with the increasing maternal age, as the females get older

they lay smaller eggs. Moreover, the egg to adult survivorship decreases, while offspring development time increases with increasing maternal age. Egg size also influences offspring performance (Fox, 1994): offspring from larger eggs develop faster and are larger than offspring from smaller eggs. However, Fox & Dingle (1994) showed that the body size of the offspring is not affected by maternal age. Larger females lay larger eggs (Fox, 1993a; Fox, 1994), but variation in egg size and offspring's body size are due to additive genetic variation and are not maternally transmitted across generations (Fox 1993b). Moreover multiple mating increase female longevity when females are nutrient stressed (Fox, 1993d), affect female survivorship (Fox, 1995), increase egg productivity (Fox, 1993d), influence both egg size and larval survivorship, but do not influence offspring development time or body size (Fox, 1993a). Females copulating with multiple males lay more eggs than those copulating repeatedly with the same male (Eady *et al.,* 2000).

Fecundity is higher in females of *Bruchidus dorsalis* that had copulated ten times than females that had copulated only once (Takakura, 1999). The fecundity of the female is often correlated with the weight of the females at emergence, in *Zabrotes subfasciatus* (Dendy & Credland, 1991) or the size and/or the elytron length in *C. maculatus* (Messina, 1991; Timms, 1998).

At the population level, differences in fecundity, patterns on egg distribution, development time and adult sizes where found among 5 populations of *Z. subfasciatus* maintained under the same conditions (Credland & Dendy, 1992). Similarly, there are differences in larval performance and oviposition preference between and within different populations of the bruchid *Stator limbatus*, depending on the host available to them in nature. These differences were found in survivorship and development time (Fox *et al.,* 1994). Fox (1993c), estimated the amount of genetic variation in oviposition preference and larval performance in two populations of *Callosobruchus maculatus*. High genetic variability was found in these parameters for the Bay Area population but not in the second one (Davis population). Moreover, the beetles from the Bay Area developed faster, but were smaller than beetles from the Davis population, tested on two host species. In their experiments, Messina (1991a, 1991b) found clear differences in the fecundity, development time and adult weight between two strains of *C. maculatus*. The Indian strain was more competitive than the Nigerian strain. Reciprocal crosses suggested an additive genetic component to these differences in competitive ability. The author assumed that variation in competitive ability was a consequence of variation in the sizes of the host seeds, typically encountered by the beetle populations. Messina & Slade (1997), investigated the mode of inheritance of host acceptance and host preference between an Asian and African populations and demonstrated that host acceptance was under the

dominance of the Asian genetic strain, whereas host preference was under an additive (intermediate) inheritance. Moreover, the egg distribution differed between an Indian and a Brazilian strain (Messina & Dickinson, 1993). In the Indian strain, females avoided adding eggs on seeds that already contained eggs.

- The environment. Guntrip & Sibly (1998), demonstrated a genotype-by-environment interaction acting on the developmental period of *C. maculatus* in relation to the temperature and humidity conditions. The relative humidity (r.h.) is a critical factor because it modifies the atmosphere in a storage environment. Under several r.h. experimental conditions, Ofuya & Reichmuth (2002) showed variability in the fitness parameters of *C. maculatus* and particularly in the number of eggs laid and the number of adults emerged from such an atmosphere. In the presence of conspecific eggs, the females of *C. maculatus* changed its behavioural pattern of oviposition (Parr *et al.,* 1996). They detected the density of eggs on the seed and were able to inhibit the oviposition (Messina & Renwick, 1985a), whereas females of *Callosobruchus chinensis* deposited a greater number of eggs on infested hosts than uninfested ones (Bhattacharya & Banerjee, 2001). In *C. maculatus* and *C. subinnotus* exists an oviposition marker which enables the females to distinguish between eggs laid and clean seeds but also allows the quantitative assessment of egg-load (Mbata, 1992a, 1992b). In the case of *Bruchidus dorsalis*, the larva itself moves and chooses to bore in a uninfested seed to avoid competition (Shimada *et al.* 2001). The availability of host seeds can also be an important factor for some strains of *C. maculatus*. Faced with a shortage of hosts, females from an Indian strain "withheld" egg laying and died without depositing 40 % of their lifetime supply (Messina, 1991).

Wilson (1994), argues that the main constraints on the oviposition behaviour of *Callosobruchus maculatus* are the amount of time available for laying eggs and the number of other females ovipositing. For Horng *et al.* (1999) the females use a threshold tactic adjusted by experience gained during the egg-laying process. Both the time since the last oviposition and the number of eggs laid by the female had a effect on the probability of accepting seeds with different number of eggs.

The goal of this study was to investigate the environmental and genetic components involved in the interaction between the bruchid *Zabrotes subfasciatus* and its host plant, within the context of the variability in the seed quality due to the domestication of the bean. To do that I addressed the following questions:

I. a) *What is the effect of seed quality on the behaviour and performance of* Zabrotes subfasciatus *?*

The hypothesis tested here is that cultivated seeds provide a better resource for the bruchids, such as a higher longevity, fecundity of the females and a greater size of the offspring.

I. b) *Are there differences in the performance and behaviour of bruchids that have been reared on wild and cultivated seeds ?*

Here I investigated if bruchids can be adapted to the seed type from which they are originated. After several generations on one type of seed (wild or cultivated), the bruchid originated from one seed type should perform better on this type of seed.

II. *Is there a genetic basis for fitness components in this bruchid ?*

Because the genotype of the insect can influence its performance, I compared the fitness traits of several families of bruchids. I hypothesized that, due to the genotypic variability, some bruchids perform better than others when they were tested on the same seed type.

Materials and method:

Effect of seed quality and bruchid origin on the behaviour and performance of *Z. subfasciatus.*

Among all of bruchid species, *Zabrotes subfasciatus* (Coleoptera: Bruchidae) is one of the main pests of field crop and stored beans in Mexico and Central America (Leroi *et al.* 1990; Ramirez- Tenorio, 1991)

I investigated the effect of the seed type (wild versus cultivated) in *Phaseolus vulgaris* (Leguminosae) on the oviposition behaviour and larval performance on individual of *Zabrotes subfasciatus*, that had been reared for 12 generations on the different bean types.

On cultivated seeds of *Phaseolus coccineus*, *Z. subfasciatus* had a shorter development time and produced larger offspring than on wild seeds (Benrey *et al*., 1998). Because wild seeds are not an optimal resource for *Z. subfasciatus*, I predicted that beetles forced to use this poor host will be "selected" for improved performance on it and are expected to perform better on it than beetles feeding on a better quality host.

Individuals of *Zabrotes subfasciatus* came from a laboratory colony reared on cowpea (*Vigna unguiculata*), at 29°C, 70% r.h., 16L:08D. Newly emerged males and females were separated in two groups. Individuals used for the first group, the "wild" treatment (W), were placed in a plastic box with wild seeds of *Phaseolus vulgaris* originated from the Malinalco locality in the state of Mexico. At each generation, fresh adults were placed on new wild seeds. The second group, the "cultivated" treatment (C), was maintained on cultivated seeds of *Phaseolus vulgaris* of the Red Kidney variety. In this group also, I used fresh cultivated seeds for each new generation. I also kept a bruchid colony on cowpea, during the same period of selection. This third group, was used as "control" (Z) treatment.

For the test, I used the 12th generation of the *Z. subfasciatus* strains. To avoid interference with the host quality of the seed used by the parental strain during the selection, I used wild seeds from another population, and cultivated seeds from another variety both of them of *Phaseolus vulgaris*. The wild seeds (w) used for the test were collected in the Tepoztlan locality in the state of Morelos (Mexico), and I used the "yellow bean" variety also called "Canary" (c) which is another cultivated variety of *P. vulgaris*, bought in a Mexican market. To avoid a misinterpretation of the oviposition behaviour due to the abrupt change of host seeds (cultivated versus wild type), I performed the test during 6 days, and thereafter analysed the changes in the performance throughout the 6 days.

More than 20 newly emerged females from each strain were randomly selected, isolated and mated in a gelatine capsule with a newly emerged male from the same group during 24 hours. During the following 3 days, all males and females from the same strain were kept in a plastic box. After this period, I isolated 20 couples in a second plastic box (∅5 cm) with 5 yellow bean seeds or 10 wild seeds. According to the seed type, I used the same number of seeds for each replicate to avoid an effect due to the seed number offered (Ceballos, 2002). Each 24h during 5 days, I changed the seeds for new fresh seeds of the same type. The 6th day, I placed twice the number of seeds in the box and left the bruchids until the female's death. One week later, I counted the total number of eggs on each bean, and the total number of beans with eggs for each box. Thus, I estimated the female's motivation to oviposit during the experiment. Subsequently, I waited for the emergence of the G1 offspring. I recorded the number of individuals, their sex, their emergence date, and the tibia size of newly emerged adults. Due to the great number of offspring obtained in particular on the cultivated seeds, only a representative sample of the emerged adults was measured..

The treatments were named with the first letter of the strain (C, W or Z) followed by the letter of the seed type used for the test (c or w). For example, the Cw group refers to bruchids from the cultivated strain (C) tested on wild seeds (w), and Zc refers to bruchids from the control group (Z: cowpea) tested on cultivated seeds (c).

I investigated the effects of the strain and the seed type on the performance of the bruchid within each treatment group (throughout their period of oviposition) and between groups (wild, cultivated and control strains). I also counted the number of seeds with eggs to investigate the patterns of distribution of the eggs.

Genetic basis for fitness components in *Z. subfasciatus*.

Using full-sib families, I investigated a possible genetic component of phenotypic variability in both oviposition and larval performance of *Z. subfasciatus*. The full-sib families of *Z. subfasciatus* were established from a laboratory population. This colony has been maintained from more than 4 years in the laboratory on black beans, a cultivated variety of *Phaseolus vulgaris*, at 29°C, 70% r.h., 16L:08D. The colony is routinely maintained by transferring several hundreds of adults to a fresh supply of black beans each time that adults have emerged from the previous supply, and it is genetically increased by including new wild individuals each year. After a previous isolation of infested beans, twenty two newly emerged females were randomly selected and individually placed in a small plastic box (internal ∅3.5 cm, external ∅ 4.5 cm), with one male during 4 days. After this period, they were allowed to oviposit on 5 black beans seeds. Each 24h, during 5 days, I renewed the 5 seeds with new fresh seeds. The 6^{th} day, I placed 10 black bean seeds and left the females until the day they died. One week later, I recorded the total number of eggs laid on each bean and waited for the emergence of the G1 offspring. I recorded the number, the emergence date and the sex of each new *Z. subfasciatus* individual. I removed the females and allowed them to mate, when possible, with their brothers during 24h hours in a gelatine capsule (full-sib family). In the case of a lack of a male brother, females were mated with males randomly chosen from the laboratory colony (half-sib family). Then, I placed the couple in a plastic box and on the 4^{th} day, I followed the same experimental procedure, renewing 5 new black beans seeds during 5 days, and on the day 6, ten seeds until the female's death. One week later I recorded the number of eggs on the beans, and the number, the emergence date, and the sex of the G2 offspring. Due to the great number of beetles obtained during this experiment, not all individuals were measured. The size of the males and females from the G0 generation were

recorded, as well as the size of all the G1 females. Then, I measured the size of a selected sample of G2 individuals originated from some G1 females, randomly chosen within the size range obtained. Because a possible effect of the oviposition day on the size and the development time (see first part of this chapter), only individuals obtained from the first oviposition day were used in the analysis.

Statistical analyses:

Effect of seed quality and bruchid origin on the behaviour and performance of *Z. subfasciatus.*

Data were tested for normality by using the Test of Composite Normality: one sample Kolmogorof-Smirnov. None of the recorded parameters met the normality assumption for parametric tests, even after transformation. Non parametric tests were used to perform the analyses. (Sokal & Rohlf, 1995)

Due to some missing group values in days 5 and 6 of the experiments, these two days were not included in the analysis. To investigate the differences between treatments, I did a Kruskal-Wallis test complemented with a Wilcoxon Rank test with a significant level (α') corrected according to the number of comparisons for each group, using the Dunn-Sidak adjustment (Sokal and Rohlf, 1995) : $\alpha' = 1 - (1 - \alpha)^{1/k}$ with $\alpha = 5\%$ and k = number of comparisons. In the case of the treatment analysis, k = 3 and $\alpha' = 0.016$. The treatments tested were: Cc, Cw, Wc, Ww, Zc, Zw, where C, W and Z refers to the origin of the bruchid, and c, w, refer to the seed type tested. So that k = 3 because for example Cc is compared to Cw (to test for the seed effect) and to Wc and Zc (to test for the bruchid strain).

When no significant differences were found between the treatments, the data were pooled and the seed type effect was tested with a Mann-Whitney U test, and the strain effect was analysed with a Kruskal-Wallis test.

The number of eggs laid on different oviposition days was analysed with a generalized linear model (glm): $f = const + \alpha_{\text{jour}} + \beta_{\text{seed}} + \gamma_{\text{origin}}$

I used S-Plus statistical program to perform these tests. (S-PLUS 2000).

The mean of the several replicates of each treatment was used for the graphs.

Genetic basis for fitness components in *Z. subfasciatus*.

For the statistical analysis, I used only data on the individuals obtained from the full-sib crosses (female G1 mated with their own brothers). Because several values in the G2 correspond to a single value of G1, I used the median and / or the mean of the data recorded in

the G2 generation. The use of a single value for each family did not influence the result of the test.

The longevity, number of eggs laid, number of offspring emerged from females of different families were analysed with a Kruskal-Wallis test (Sokal & Rohlf, 1995).

Correlations between the different parameters were analysed with the significant test of correlation coefficient of Pearson (Pearson's product – moment correlation). Due to some small sample sizes, some family were not used for the statistical analysis in the number of eggs laid and offspring emerged. Also, for the larval development time, due to the small sample size, I pooled the development time in categories. This did not influence the result of the statistical tests performed with a Pearson's chi-square test.

The tests were performed with the S-Plus statistical program. (S-PLUS 2000). The mean of the several replicates of each treatment was used for the graphs.

Results:

Effect of seed quality and bruchid origin on the behaviour and performance of *Z. subfasciatus.*

Comparisons within treatments throughout the oviposition period.

The analysis of the effect of the oviposition day on the different parameters showed significant variations mostly between the first, two and the last two days of oviposition. The main variations were found in the number of beans used ($0.0001 < p < 0.35$), the number of eggs laid (and therefore the number of offspring that emerged) ($0.001 < p < 0.01$), the development time of males ($0.001 < p < 0.004$) and females ($0.001 < p < 0.005$). The male tibia size is not influenced by the day of oviposition ($p > 0.12$) whereas there is a significant effect of the day of oviposition on the female size for half of the treatments. The secondary sex ratio is also not influenced by the oviposition day.

Because most of the eggs were laid during the first day of oviposition, I used the values of this day for the analysis between treatments.

Comparisons between the treatments.

Female longevity:

Figure 1a shows the number of females alive through time, for the different treatments. I found a significant effect of the seed type on female longevity for the three strains of bruchid. When a female laid eggs on wild seeds, its life was prolonged than when oviposited on cultivated seeds ($0.0001 < p < 0.0003$; Fig. 1b). There were no significant difference between the strains when bruchids were tested on wild seeds ($0.028 < p < 0.35$). But significant differences were found on cultivated seeds, between the wild and the control strain ($p = 0.0007$). Females that originated from the control strain (from cowpea) live longer than females that originated from wild seeds of *P. vulgaris*. The difference between these both strains in their longevity is close to being significant on the wild seeds ($p = 0.028 > 0.016$).

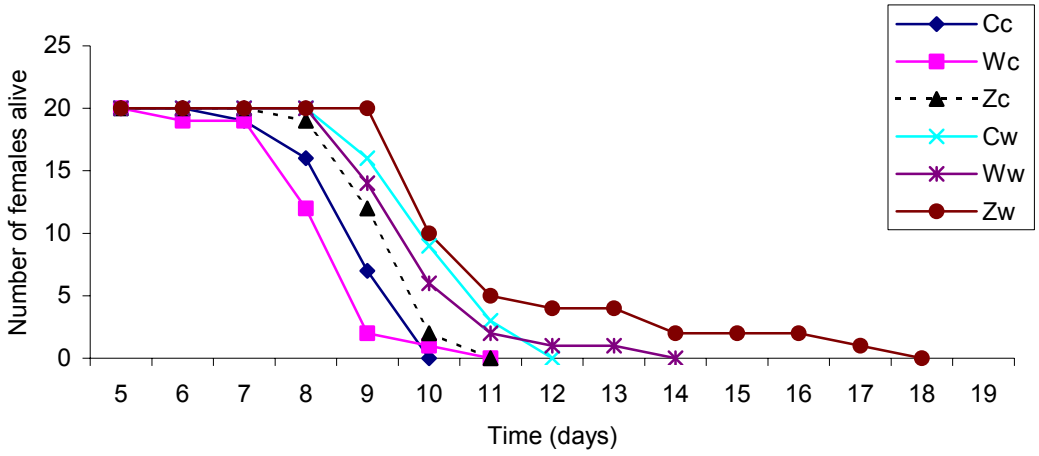


Figure 1a: Number of females alive through time for the different treatments (bruchid strains: C: cultivated, W: wild, Z: control; and seed type: c: cultivated, w: wild).

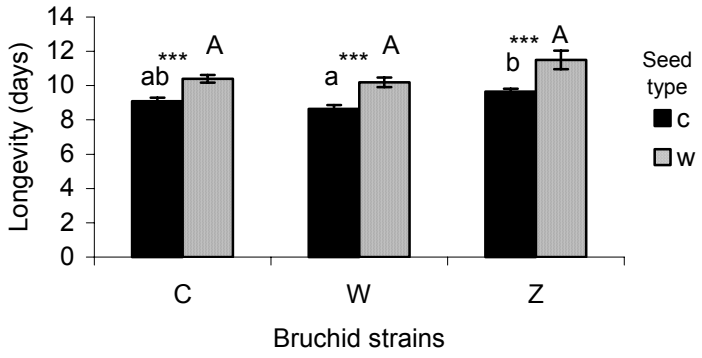


Figure 1b: Effect of the seed type (c: cultivated, w: wild) and the bruchid strains (C: cultivated, W: wild, Z: control) on the longevity of the females (days). Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds) (n = 20).

Percentage of females that laid eggs:

The analysis of the statistical model according to the observed values during the experiment (Fig. 2) showed that the seed type and the strain have an influence on the number of female that laid eggs which is lightly greater on wild seeds than on cultivated seeds ($p = 0.047$; Table 1). This can be explained by the fact that females live longer on wild seeds, and therefore can oviposit during a longer period time. A significant strain effect is found between the females from the wild strain which oviposited less than the females of the cultivated and the control strains ($p = 0.0001$; Table 1). Moreover, there is a clear decrease through time in the number of females ($p < 0.001$; Table 1).

				SE	P values
const	3.9			0.32	<0.0001
α	-0.76			0.074	< 0.001
β	c: -0.202	w: 0.202		0.1	0.047
γ	C: 0.23	W: -0.56	Z: -0.33	0.14	0.0001

Table 3: Values of the generalized linear model $f = const + \alpha_{\text{jour}} + \beta_{\text{seed}} + \gamma_{\text{origin}}$ for the number of oviposition.

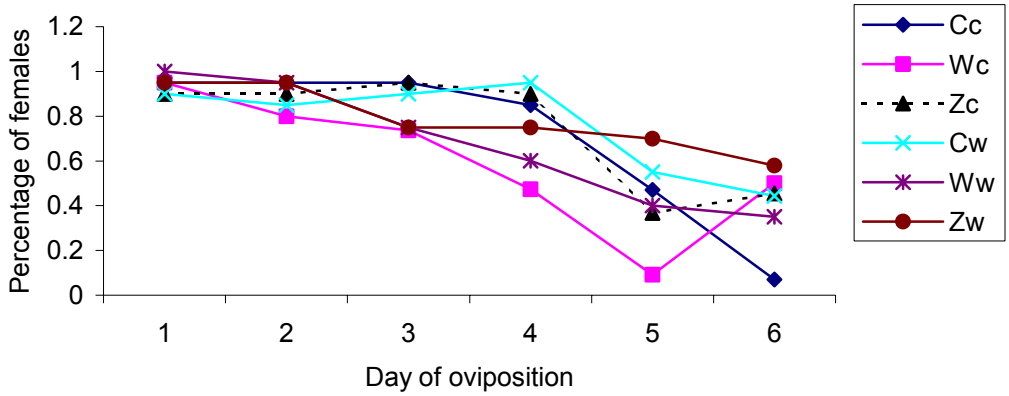


Figure 2 : Percentage of females that laid eggs through time (bruchid strains: C: cultivated, W: wild, Z: control; and seed type: c: cultivated, w: wild) (data observed: $2 < n < 20$).

Number of eggs/ number of offspring:

Because the number of offspring is correlated with the number of eggs laid by the females, and show the same pattern, I present here the differences for the number of eggs, and mention the differences in the offspring analysis only if they differ from the number of eggs.

Figure 3a shows the variation in the number of eggs laid by the females through the time. Females laid more eggs on the first day, and the number of eggs laid decreased during the experimental period, which corresponds with females getting older ($0.001 > p$; Fig.3a). Females in the Cw treatment laid the same number of eggs throughout the whole period of oviposition ($p = 0.19$).

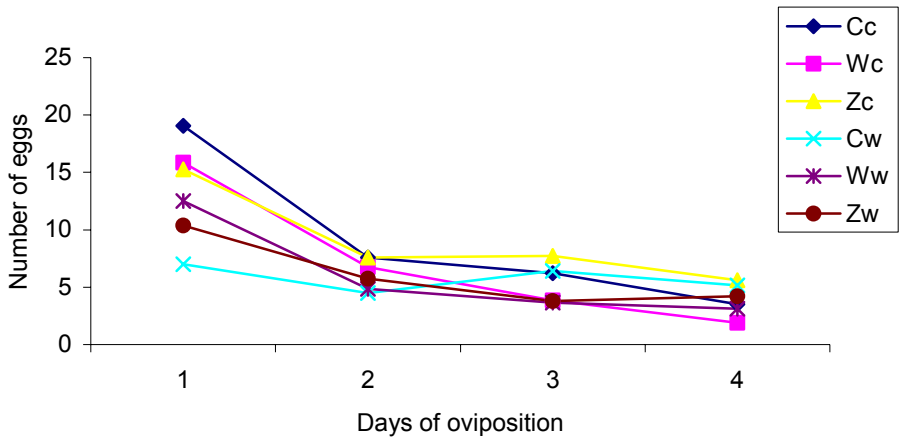


Figure 3a: Number of eggs laid by the females through time ($19 < n < 20$). Treatments: bruchid strains: C: cultivated, W: wild, Z: control; and seed type: c: cultivated, w: wild.

The total number of eggs laid varied with the seed type for the cultivated strain ($p < 0.001$) and for the control strain ($p = 0.014$) (Fig. 3b). For these strains, the total number of eggs oviposited was greater on cultivated seeds than on wild seeds. Females from the wild strain deposited the same number of eggs on wild seeds as on cultivated seeds ($p = 0.40$). The number of eggs laid changed with female age and the seed type did not have the same effect through time. For example, on the first day of oviposition the seed type affected the number of eggs laid only for the cultivated strain ($p < 0.001$); whereas on the third day, the significant effect of the seed type is found only for the control strain ($p < 0.0001$).

There were significant differences on the total number of eggs laid between the strains on the cultivated seeds. When cultivated seeds were offered, females from the wild strain laid fewer eggs than females from the other strains (Wc vs Cc: $0.001 < p$; Wc vs Zc: $p = 0.0002$; Fig.3b). No significant differences were found on wild seeds between the strains on the total number of eggs laid ($0.55 < p < 0.7$; Fig. 3b), except during the third day, in which females from the cultivated strain oviposited a greater number of eggs than the two others strains (Cw vs Wc: $p = 0.006$; Cw vs Zw: $p = 0.0018$). Moreover, during the third and the fourth experimental days, females of the wild strain oviposited fewer eggs than females originated from the cultivated strains ($0.0046 < p < 0.0098$). But at the emergence, differences between the wild and the cultivated strain disappear, and the same number of offspring emerge from the wild and the cultivated strain on cultivated seeds ($p = 0.098$), which means that survivorship is higher for the wild strain. It appears that on cultivated seed, the wild strain have a greater success in their egg-to-adult survival rate.

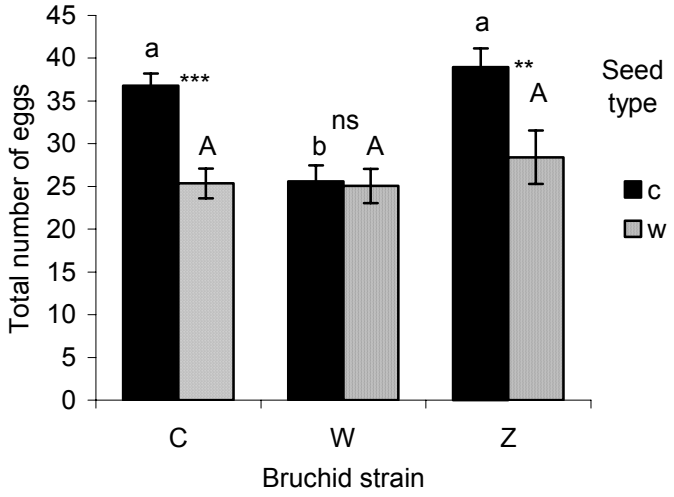


Figure 3b: Effect of the seed type (c: cultivated, w: wild) and the bruchid strain (C: cultivated, W: wild, Z: control) on the total number of eggs laid. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds).

Secondary sex ratio:

The sex-ratio was estimated by recording the sex of emerged adults. It did not show any difference according to the day of oviposition of the eggs, except the last day for the Wc treatment where the sex ratio is female biased (0.22) and different to the one found with offspring emerged from eggs laid on the other days ($p = 0.018$).

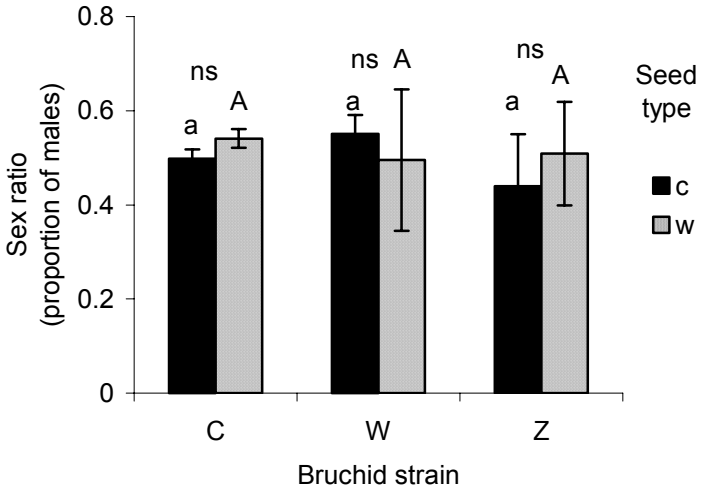


Figure 4a: Effect of the seed type (c: cultivated, w: wild) and the bruchid strain (C: cultivated, W: wild, Z: control) on the secondary sex ratio represented as male proportion. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds) ($19 < n < 20$).

Because the analysis between the treatments did not reveal differences due to the seed type or the strain ($0.075 < p < 0.30$) (Fig. 4a), data from all treatments were pooled. The total sex ratio (calculated for all the experimental days) was influenced by the seed type ($Z = -13.37$; $p < 10^{-4}$; Fig. 4b), but not by the strain origin ($\chi^2 = 3.07$; $p = 0.22$). The sex-ratio is slightly male biased on the wild seed (0.51 +/- 0.016), whereas it is not different from 1:1 on cultivated seeds.

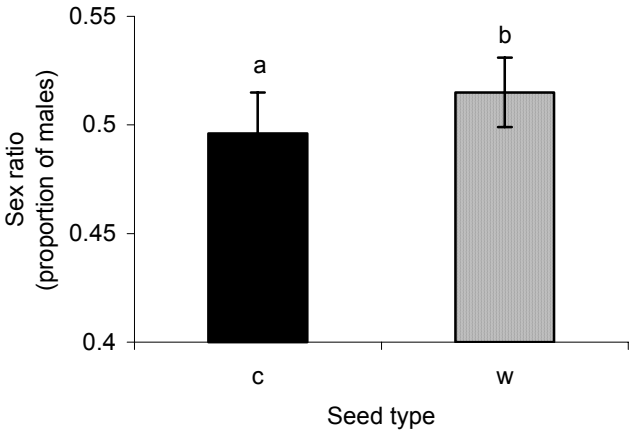


Figure 4b: Effect of the seed type (c: cultivate, w: wild) when all the data are pooled ($n = 60$). Different letters indicate significant differences

Development time:

Depending on the day on which eggs were laid, bruchids had a different development time (males: Fig. 5a; females: Fig. 5b). Individuals from eggs laid the first day emerged earlier than the others except for individuals from the Cw group where this was not found. To achieve their development, males needed between 26.9 +/- 0.1 to 30.6 +/- 0.4 days. Females needed more time with values between 27.5 +/- 0.1 to 30.9 +/- 0.4 days.

For both sexes, the larval development time is affected by the seed type and the bruchid strain. For all strains, individuals from the wild seeds developed slower ($0.001 < p < 0.0043$; males: Fig. 6a; females: Fig. 6b). Moreover, the strain had a significant effect on the development time on cultivated seeds but not on wild seed ($0.23 < p < 0.76$). When emerged from cultivated seeds, offspring from the cultivated strain developed slower than offspring from the two others strains (males: Cc vs Wc: $p < 0.001$; Cc vs Zc: $p = 0.0003$; Fig. 6a; females: Cc vs Wc: $p < 0.001$; Cc vs Zc: $p < 0.001$; Fig. 6b).

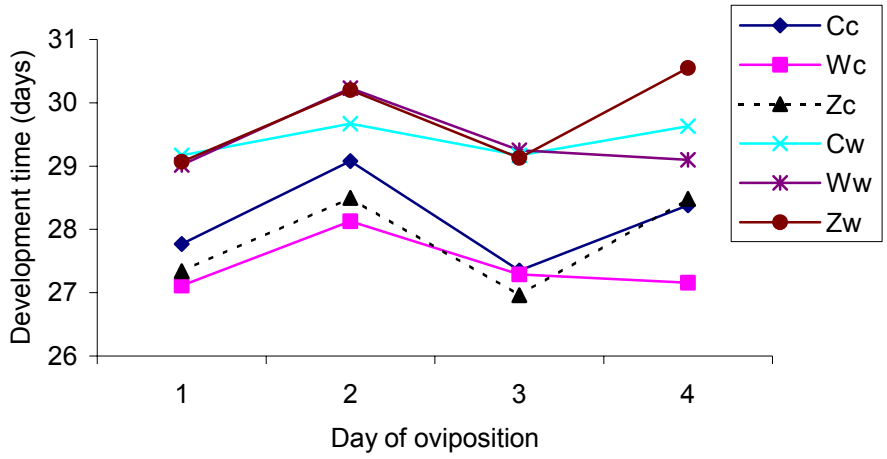


Figure 5a: Effect of bruchid strain (C: cultivated, W: wild, Z: control) and seed type (c: cultivated, w: wild) on the larval development time of the male offspring through time ($6 < n < 155$).

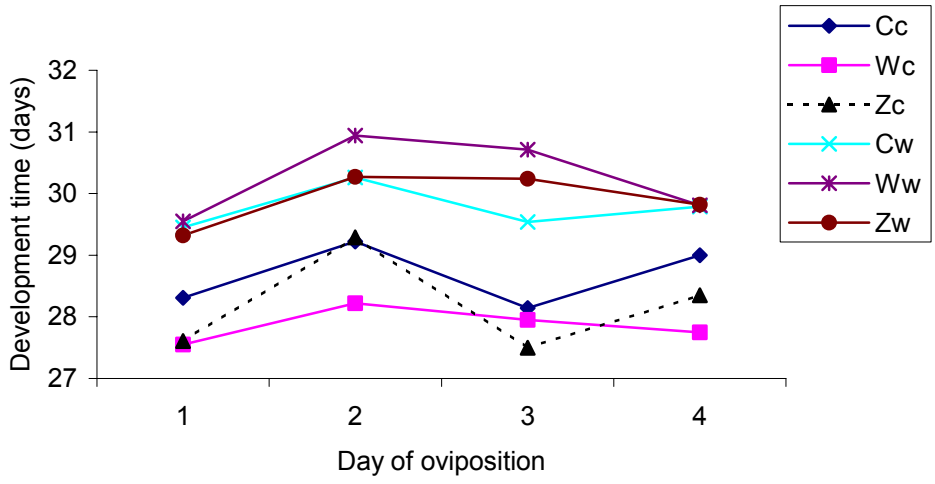


Figure 5b: Effect of the bruchid strain (C: cultivated, W: wild, Z: control) and the seed type (c: cultivated, w: wild) on the larval development time of the female offspring through time (12 < n < 170).

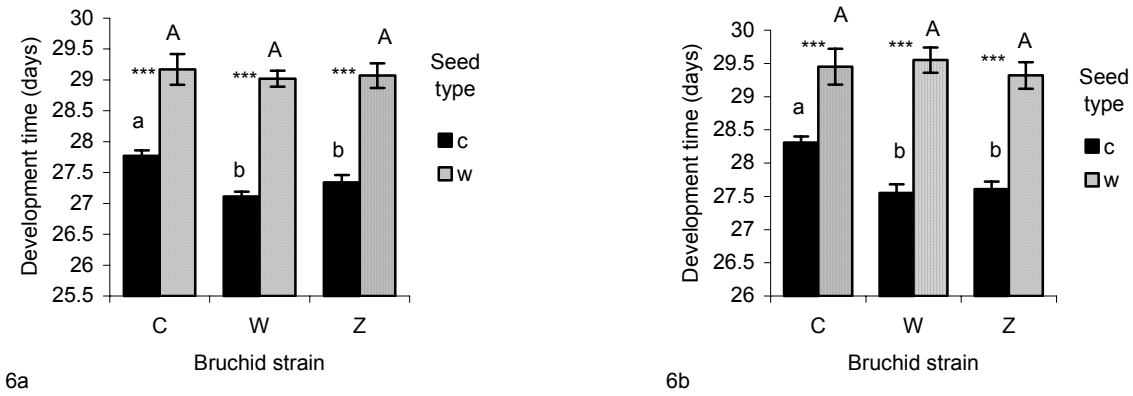


Figure 6a: Effect of the seed type (c: cultivated, w: wild) and the bruchid strain (C: cultivated, W: wild, Z: control) on the larval development time of the males. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds). ($6 < n < 155$).

Figure 6b: Effect of the seed type (c: cultivated, w: wild) and the bruchid strain (C: cultivated, W: wild, Z: control) on the larval development time of the females. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds). ($12 < n < 170$).

Tibia length:

Zabrotes females are bigger than males. The tibia size of males varied between 26.8 +/- 0.0.31 mm to 28.41 +/- 0.17 mm, whereas for the females, between 30.74 +/- 0.32 mm to 33.74 +/- 0.17 mm. The tibia size did not change according to the oviposition day for the males, except for the Cw group (Fig. 7a), whereas it was influence by the oviposition day for females (Fig. 7b).

For males, the seed type had a significant effect only on males originated from the cultivated strain. Males emerged from the cultivated seeds are larger than males that emerged from the wild seeds ($p = 0.005$) (Fig. 8a). On cultivated seed, males from the control strain are larger than males from the wild strain ($p = 0.0065$), whereas on wild seeds, males of the control strain are larger than males originated from the cultivated strain ($p = 0.0001$) (Fig. 8a).

For females, there are significant differences due to the seed type and the bruchid strain (Fig. 8b). For the cultivated and the wild strains, females that emerged from cultivated seeds were larger than females that

emerged from wild seeds ($0 < p < 0.0016$). Moreover, on cultivated seeds females from the wild strain were smaller than the females from the two others strains ($0.001 < p$). On wild seeds, the females from the control strain were larger than the females from the two others strains ($0.001 > p$) (Fig.8b).

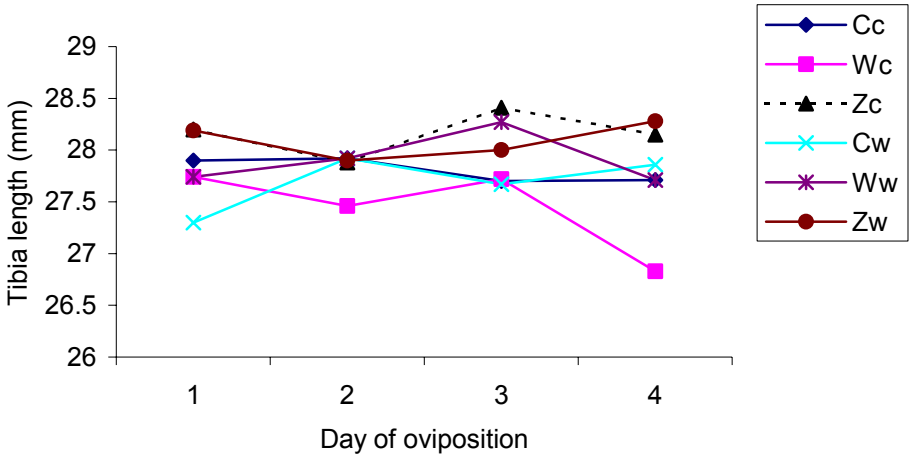


Figure 7a: Effect of bruchid strain (C: cultivated, W: wild, Z: control) and seed type (c: cultivated, w: wild) on the tibia length of the male offspring through time ($6 < n < 72$).

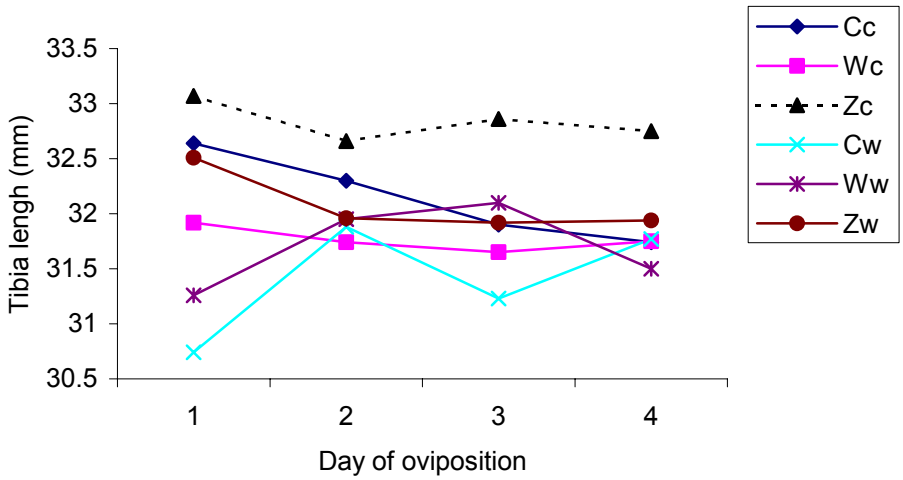


Figure 7b: Effect of bruchid strain (C, W, N) and seed type (c, w) on the tibia length of the female offspring through time ($12 < n < 77$).

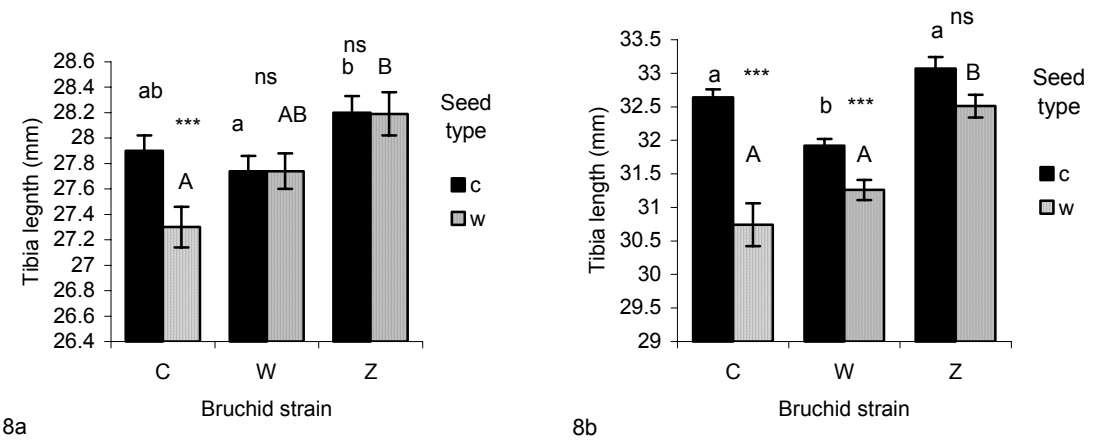


Figure 8a: Effect of the seed type (c: cultivated, w: wild) and the bruchid strains (C: cultivated, W: wild, Z: control) for the first day on the tibia size of the males. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds) ($6 < n < 72$).

Figure 8b: Effect of the seed type (c: cultivated, w: wild) and the bruchid strains (C: cultivated, W: wild, Z: control) for the first day on the tibia size of the females. Asterisks indicate differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds) ($12 < n < 77$).

Number of seeds with eggs:

Many of seeds offered to the bruchids were not carrying eggs. I analysed separately the influence of the bruchid strain on the number of seeds (wild or cultivated) used to oviposit. Figure 9a shows the number of seeds with eggs through time. There was a clear decrease in the number of beans used for oviposition through time for most treatments ($p < 0.0001$), except for the groups Cw ($p = 0.35$) and Zc ($p = 0.21$). The number of seeds on which females from these two groups laid their eggs was not significantly different during the 4 days of the experiment (Fig.9a).

When wild seeds were offered, there was no significant difference between the strains in the total number of beans used ($0.60 < p < 0.89$; Fig. 9b). Significant differences between the strains were found when cultivated seeds were offered. Females from the wild strain used less cultivated seed than the females from the two other strains (Wc vs Cc: $p = 0.016$; Wc vs Zc: $p = 0.0004$; Fig. 9b).

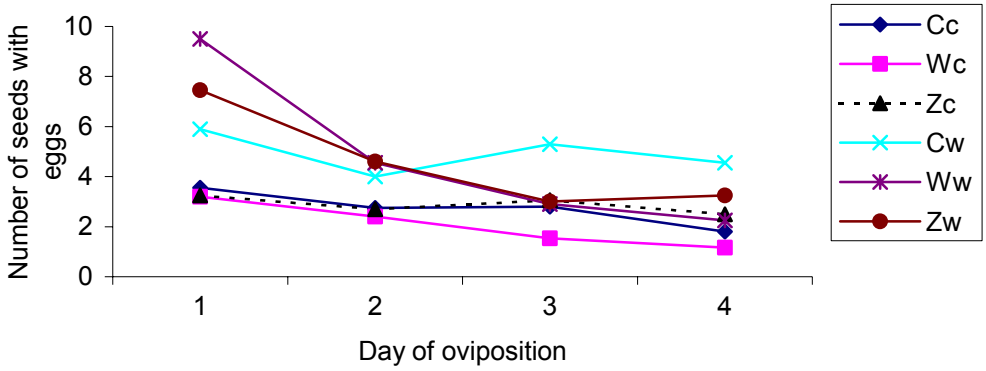


Figure 9a: Pattern of distribution of the eggs during the different days of oviposition (19 < n < 20) (Bruchid strain: C: cultivated, W: wild, Z: control; Seed type: c: cultivated, w: wild).

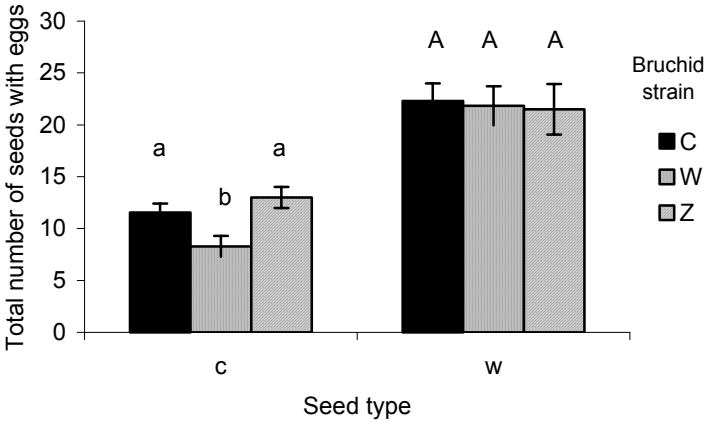


Figure 9b: Effect of the bruchid strain (C: cultivated, W: wild, Z: control) and the seed type (c: cultivated, w: wild) on the number of seeds with eggs. Different letters indicate significant differences between the strains (a,b: on cultivated seeds; A, B: on wild seeds). (19 < n < 20).

Genetic basis for fitness components in *Z. subfasciatus*.

Longevity:

Because the female is the sex that oviposits the eggs, I only investigated the longevity of female. The analysis of the longevity of the G1 females revealed significant differences between families ($\chi 2 = 30.09$; df = 15; p = 0.012; Fig. 10). Depending on their family, some females live longer than others. For example, the females of the families 2 and 17 showed the shorter longevity, in comparison to the females of families 4, 11 and 18. But there was no correlation between the longevity of these females and the longevity of their mothers (t = -0.62; df = 138; p = 0.534). The difference in the longevity is not correlated with the size of the

female (p = 0.92), the number of eggs laid (p = 0.24), or the number of emerged offspring (p = 0.38), nor the secondary sex ratio (p = 0.72).

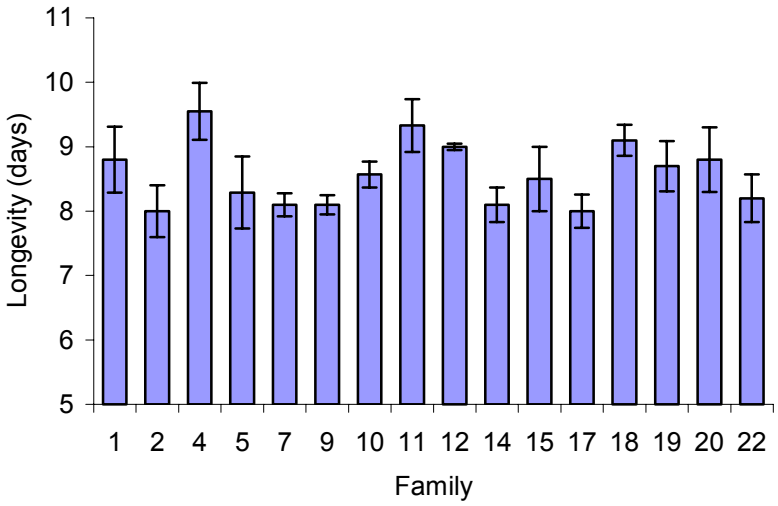


Figure 10: Longevity of the females G1 of different families (4 < n < 18).

Fecundity:

There is a significant difference in the number of eggs laid by the females according to their family (t = 39.11; df = 15; p = 0.0005) (Fig. 11). There is also a significant difference in the number of offspring that emerged (t = 40.64; df = 15; p = 0.0004) (Fig. 12). For example females from family 10 laid twice as many eggs as females from family 5. There was no correlation between the number of eggs laid by the mother (G0) and the number of eggs laid by their daughters (t = 1.15; df = 144; p = 0.25). Also, no correlation was found between mothers and daughters in the number of emerged offspring (t = 0.97; df = 156; p = 0.33).

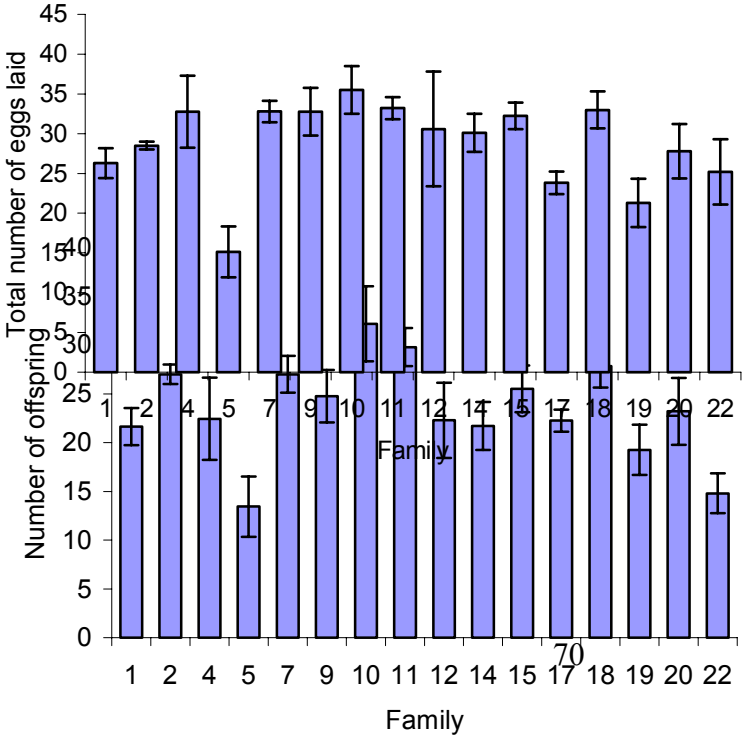


Figure 11: Total number of eggs laid by the female G1 of different families (4 < n < 18).

Figure 12: Total number of emerged offspring in G2 of different families ($4 < n < 18$).

Sex-ratio:

The emerging sex ratio from the females G1 is positively correlated with the sex-ratio emerging from eggs laid by their mothers ($t = 2.79$; $df = 18$; $p = 0.012$). Females that laid eggs that produced a sex ratio biased toward females, had daughters that also laid eggs which produced a greater number of females (Fig. 13). One could suggest that when the female oviposits a greater number of eggs, the resources available for each egg decrease and differentially mortality occurs, resulting in a biased sex ratio. However, the sex ratio produced by the G1 females was not correlated with the number of eggs laid by these females ($t = -0.34$; $df = 140$; $p = 0.73$), and it is not dependent on their size ($t = -0.29$; $df = 36$; $p = 0.77$).

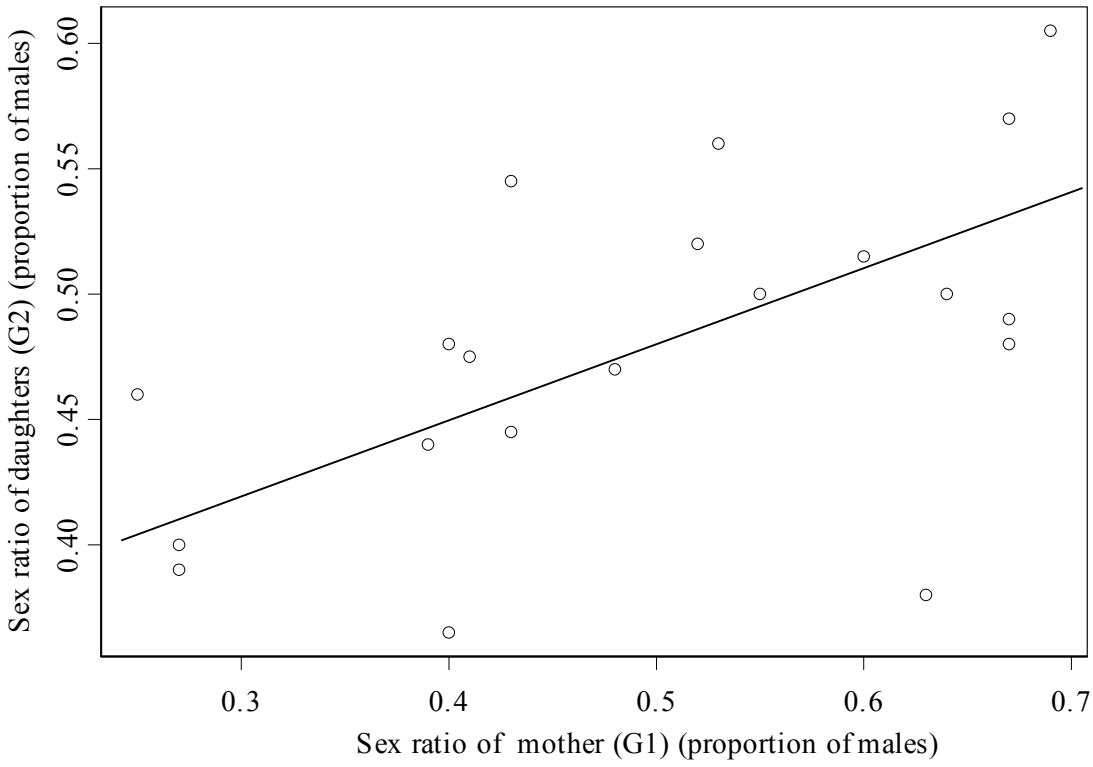


Figure 13: Relationship between the secondary sex-ratio (proportion of males) of the progeny of mother and daughters of different families (n = 19). Each point represent the median of one family.

Larval development time:

The analysis of the larval development time of the males and females of the G2 generation, showed strong significant differences according to their family (males (Fig.14): $\chi^2 = 163.88$; df = 57; $p < 0.0001$; females (Fig.15): $\chi^2 = 253.63$; df = 76; $p < 0.0001$). Offspring originated from the families 2, 10 and 12 emerged earlier than others, such as, offspring from the families 1, 4, 15 and 19. There was a significant negative correlation between the size of the males G2 and their development time ($t = -2.79$; df = 36; $p = 0.008$), whereas there was no correlation in the G2 generation between the size of the female and their larval development time ($t = -1.61$; df = 35; $p = 0.12$). The males that develop faster are larger (Fig. 16).

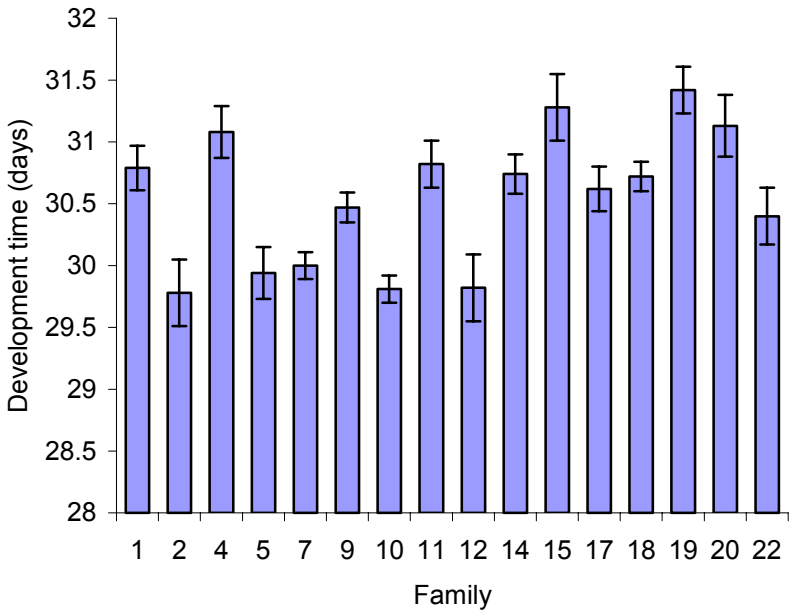


Figure 14: Larval development time of males G2 of different families (25 < n < 147).

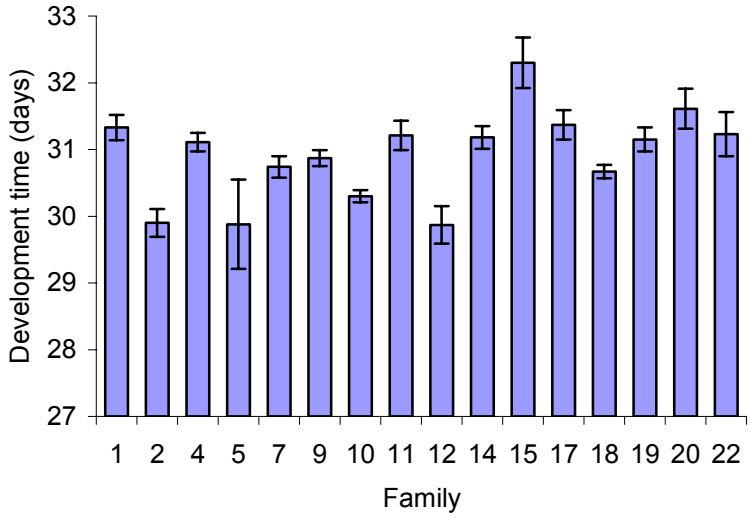


Figure 15: Larval development time of females G2 of different families (20 < n < 180).

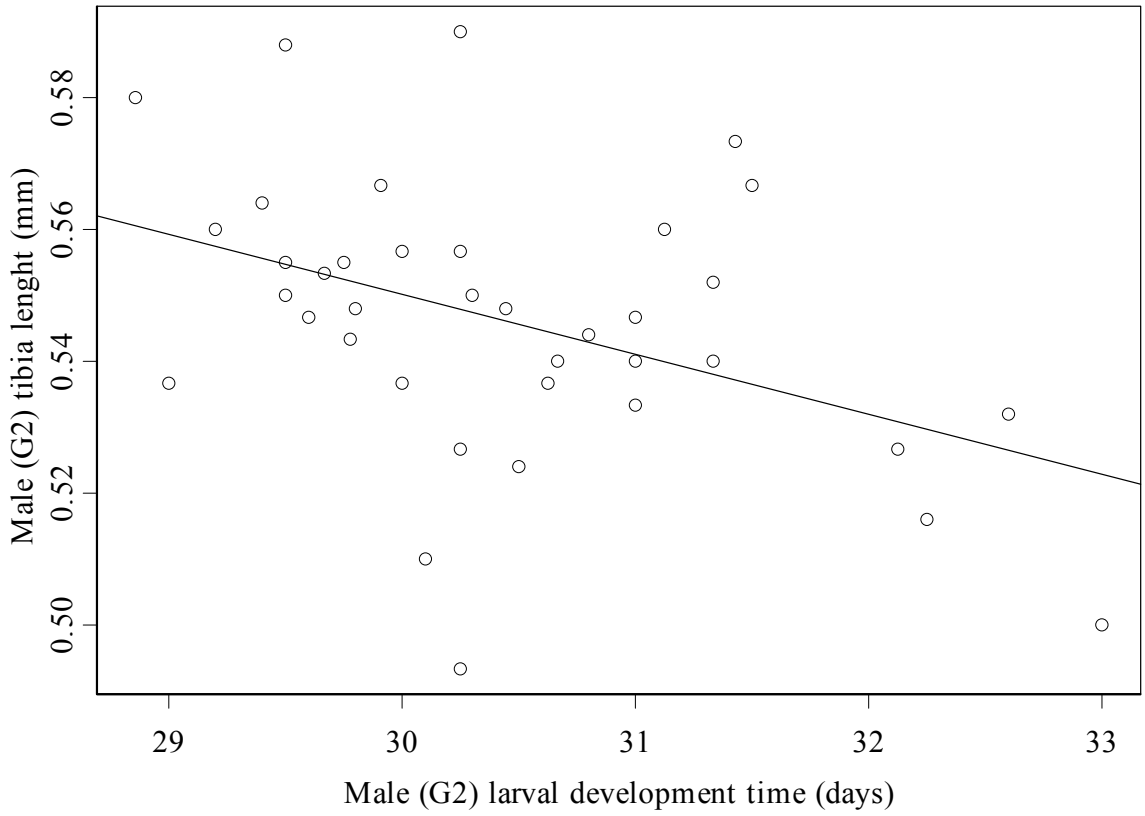


Figure 16: Relationship between male tibia length and male development time for individuals of G2 generation (n = 37) Each point represent a mean for one family.

Size:

There was no correlation between the size of the G0 mothers and the size of their offspring in the G1 generation (males: $t = 1.57$; $df = 143$; $p = 0.12$; females: $t = -1.3$; $df = 169$; $p = 0.19$). But there was a strong correlation between the size of the G1 females and the size of the offspring of the G2 generation (males: $t = 7.92$; $df = 194$; $p < 0.0001$; Fig. 17; females: $t = 11.03$; $df = 215$; $p < 0.0001$; Fig. 18). Small females produced small offspring. There was also no correlation between the size of the offspring and the sex ratio from which they are originated (males: $t = -1.62$; $df = 188$; $p = 0.10$; females: $t = -0.59$; $df = 208$; $p = 0.55$).

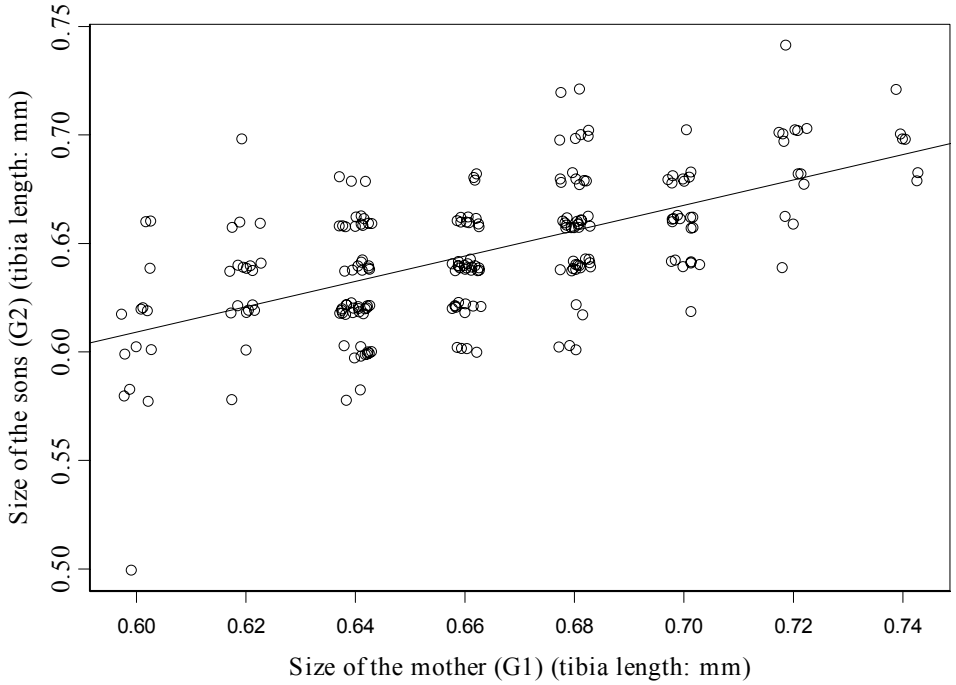


Figure 17: Relationship between the size of the females (G1) and the size of their male offspring (G2) (tibia length, mm) (n = 195).

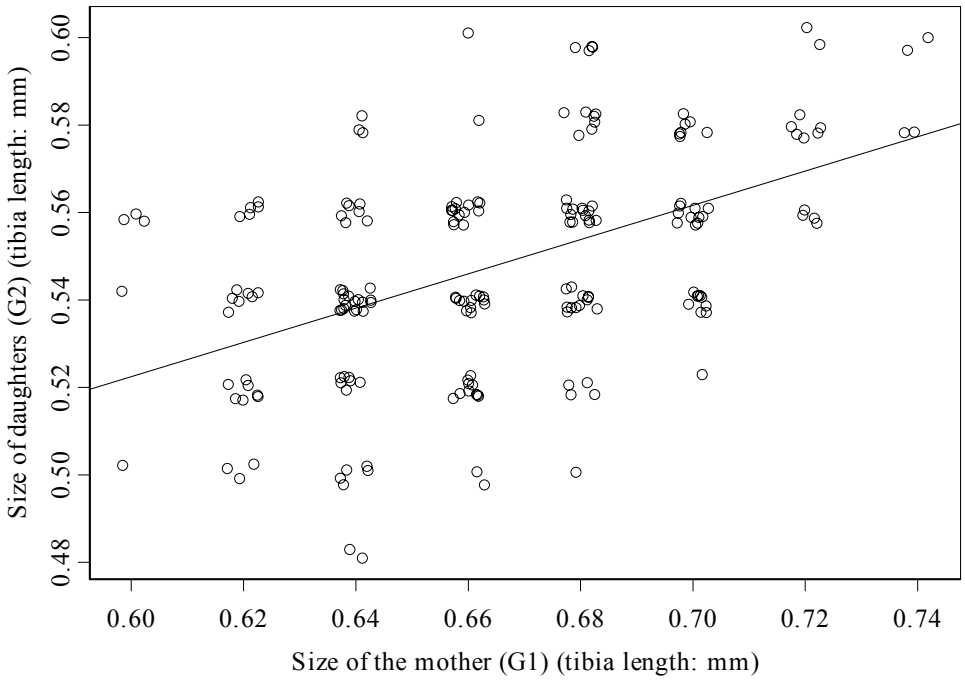


Figure 18: Relationship between the size of the females G1 and the size of their female offspring (G2) (tibia length, mm) (n = 216).

Number of seeds with eggs:

There was no correlation between the number of seeds with eggs oviposited by the females G0 during the test and the number of seeds with eggs oviposited by their daughters ($t = 0.31$; $df = 18$; $p = 0.76$).

Discussion:

Effect of seed quality and bruchid origin on the behaviour and performance of *Z. subfasciatus.*

The results of this experiment revealed and important influence of the seed type and the bruchid strain on all the life history traits examined. The first hypothesis for this experiment was that cultivated seeds could provide a better resource for the development of the bruchids than the wild seeds. Cultivated seeds of *Phaseolus coccineus* were shown to be a better resource for bruchids than wild relatives (Benrey *et al*., 1998). Here I found the same results for *Phaseolus vulgaris*. On cultivated seeds, females originated from the cultivated seeds had a higher fecundity than females originated from the wild seeds and this was not due to differences in longevity which could allow some females to lay their eggs during a greater period. Moreover, the better quality of the cultivated seed was also demonstrated with the fact that individuals that emerged from wild seeds had a longer development time than individuals that emerged from cultivated seeds.

Plaza (2001) showed that longevity of females of *Z. subfasciatus* varied with the substrate of oviposition offered to the females, the shorter longevity was found with normal seeds, in comparison to seeds in which the seed coat was removed or glass marbles. I also found an effect of the seed type on the longevity of the females. Females that laid eggs on cultivated seeds had a shorter longevity than females that laid eggs on wild seeds. Longevity can be related to a cost of reproduction because these females have more offspring. However, this is not the case with the "wild" strain in which the females have the same number of offspring that emerged from wild or cultivated seeds, whereas their longevity is influenced by the seed type. Moreover, females from the control strain (Z) had the higher longevity, but they did not produce more offspring than the other strains. In this case I argue that cowpea (*Vigna unguiculata*) represents a better host plant for the females which did not suffer from the cost of reproduction. Another possibility is that these females are not so sensitive to the allelochemicals present in the *Phaseolus* seeds which can increase female mortality independently of their reproduction. Fox and Tatar (1994) showed with the bruchid *Callosobruchus maculatus* that the presence of host seed influence the mortality rate, which varied between the host seed species used (*Vigna angularis* and *V.*

unguiculata). These differences in mortality were not explainable by differences in reproductive rates, which suggests that allelochemicals contribute to the mortality, and confound the hypothesis of a trade-off between reproduction and mortality. In my experiment, I know that domestication has altered the allelochimical diversity and concentrations in the *Phaseolus* seeds, but I do not know to what extent these changes can affect the fecundity and longevity of the bruchids.

One interesting aspect that emerges from these results, is the effect of adaptation on the bruchid's performance. This confirm the second hypothesis of this chapter. After 12 generations on the wild seeds, it appears that individuals that originated from the wild strain assimilated the nutrients better than the individuals originated from the cultivated strain. On cultivated seeds which provide a better host plant than their wild counterparts, the "wild" individuals developed faster than the "cultivated" individuals but emerged with the same size. Moreover, there was no difference on wild seeds which suggests that the two strains are affected by the poor host quality in a similar way, and the individuals from the wild strain did not perform better on the wild seeds. Therefore, the host plant is also an important factor in the success of an artificial selection process. Tucic *et al.* (1995) found an effect of the selection on the host utilization by *Acanthoscelides obtectus* after 35 generations on *Cicer arietinum* but not on *Phaseolus vulgaris*. Perhaps varieties of the host plant *Phaseolus vulgaris* used for our experiment are not variable enough to induce a selection on our *Z. subfasciatus* strains. Moreover, as Timms suggests (1998): "natural selection is expected to produce organisms that are well adapted to their normal environment, this does not mean that they will never have a higher fitness when transferred to a novel environment". This could be the case with *Z. subfasciatus*, where individuals from the wild strain develop better on cultivated seeds.

An unexpected result was the finding on variation in the development time and size of the offspring that emerged from eggs laid during two successive days. For other insects such as, *Epirrita autumnata* (Lepidoptera), the order in which eggs are laid by the female has also a significant effect on the development of the larvae: first hatched larvae grow larger than the ones that hatch last (Ruohomaki *et al.*, 1993). The differences between groups seems to indicate a difference in the quality of the eggs laid by the females as it was found for other bruchid beetles (Fox, 1994; Fox & Mousseau, 1996). For *Zabrotes subfasciatus*, a previous study showed a great variation in the egg size from 0.16 mm to 0.32 mm (Plaza, 2001). In my study, offspring originated from the first oviposition days emerged earlier and were larger than offspring originated from the second oviposition day. One

explanation may be that bruchid females after being kept for four days without host plants, laid the greatest number of eggs once they were in contact with the seeds, and females maximized the quality of these first eggs to ensure the good development of their offspring. The second day, the female is "tired", less motivated and the quality of the eggs decreases. In this study I did not measure the size of the eggs laid by the females of *Z. subfasciatus* for the different treatments. I also did not consider in the analyses the density of larvae inside the seed, which may affect the larval development due to a possible competition for resources. But Dendy & Credland (1991) demonstrated that for *Z. subfasciatus*, the larval density did not influence the adult weight which is correlated with the fecundity.

However, the results also showed that males from wild seeds need more time to achieve their development but obtain a similar size to the one of males originated from the cultivated seeds. As mentioned by Fox (1993a), the fact that some bruchid developing longer to pupate at the same size than bruchids from another origin, in his experiment from eggs laid later than other, suggest that size, rather than development time, may be a cue as to when larvae should pupate. My experiment agree this theory with males. The females are more sensitive to the host seed quality because on wild seed (the poor host), they needed more time to develop and were smaller than females from cultivated seeds. These differences between males and females could be explained by the fact that because males are smaller, they may require less resource than females. Therefore they can develop on wild seeds by feeding during a greater period than on cultivated seeds, to obtain all the nutrients required for their successful development, whereas females, with a larger quantity of resource needed, cannot feed ad libitum on wild seeds, and have to emerge with a smaller size.

Another interesting point is that although females from the cultivated strain oviposited more eggs during the last days of the experiment than females from the wild strain, these two strains have the same number of emerged offspring. Although it appears that fecundity of the "cultivated" females is higher than that of the females from the wild strain, perhaps as a result of more and better resources accumulated during their larval development, the egg quality of

the cultivated strain did not last more than three days, and thereafter the larval success decreased, leading in comparison to a higher survivorship for the eggs of the females from the wild strain. It would be interesting to study the fitness parameters of such strains in the case of competition for the seed resources, and to check if offspring from females from the cultivated strain develop better during the first oviposition days but are replaced for offspring of the wild strains in the last days. This will allow us also to investigate if, as for *Stator limbatus* for which the development time of the larvae is influenced largely by a maternal effect, the larvae can adjust the length of their development period to compensate for variation among mothers (Fox, 1998).

For several parameters (number of eggs, number of offspring, male size and development time of the individuals of both sexes), the Cw group (individuals from the cultivated strain tested on wild seeds) did not show the same variability than the other treatments during the successive days of the experiment. I believe that these effects could be due to the strong host plant change. For this group, the transfer from a good host plant (cultivated seeds) to a poor host plant (wild seeds) seems to have a stronger effect than the transfer to a different host plant species such as for the control strain where females originated from *Vigna unguiculata* and were tested on *Phaseolus vulgaris*.

Genetic basis for fitness components in *Z. subfasciatus*.

All the bruchid families were reared under the same conditions. Therefore, between family differences must have a genetic basis. The loss of genetic variability in bruchid population can occur in only three generations of laboratory rearing (Fox, 1993c). This lack of variability seems not to be the case in the laboratory population of *Z. subfasciatus* and genetic variability was found between females originated from the same colony despite many generations in the laboratory.

My hypothesis on the better performances according to the genotype was checked in this experiment because between family variations were found in longevity, fecundity, sex ratio, and larval development time. All of these parameters are important in insect fitness. Even if the longevity was not correlated with a greater fecundity, a longer life span allows the females to search for more suitable hosts for a longer period of time, a better host plant

species, or seeds not previously infested by other bruchids. As it is the case for others bruchid species (Messina & Renwick, 1985a, 1985b) females of *Z. subfasciatus* avoid to lay their eggs in the presence of conspecifics (Plaza, 2001). The fecundity is also dependent on the genotype. Some females laid more eggs than others. We may assume that as for *Callosobruchus maculatus* (Messina, 1991; Timms, 1998), the fecundity is directly related to the size of the females, larger females lay more eggs (Callejas, 1996), but it was not the case in this experiment. However, Dendy & Credland (1991) found in *Z. subfasciatus* that fecundity was related to the females weight. In the case of the larval development time it was highly variable for both sexes depending on their family of origin. It is important because females with offspring that develop faster than the others, are able to increase the number of generations and thus, may be able to better spread their genotype.

One non expected result was that males that developed slower were smaller. This was also the case with the bruchid *Callosobruchus subinnotatus* (Mbata, 1993), where high larval density prolonged the developmental period and caused the production of "miniature" individuals. However, it is usually known that a bruchid can counterbalance a lower food quality by a longer larval development time inside the seed (Benrey *et al.,* 1998). In *Stator limbatus*, and *S. pruininus*, larvae adjust the length of their development period to compensate for variation among mothers (Fox, 1998; *Fox et al*., 1999). In my experiment I could not exclude an artefact due to the density of bruchid larvae in the seeds offered, but this still would not explain the specific effect on males and not on females.

The genetic variability between the families was clear in the comparisons of individuals from the first and second generation (G1 and G2) (full-sib families) but not with the generation of origin (G0). For example, there is a significant correlation for body size between G1 and G2, for both sexes, whereas this correlation did not exist between the generation G0 and G1. Then, we cannot assume that this trait is originated from one single

parent, or from both. The relative weight of each parental genome can be investigated by the analysis of the performance of the half-sib offspring obtained with females originated from the same families used in this experiment, but mated by males randomly chosen from the laboratory colony.

In conclusion, this experiment confirms the importance of the quality host plant species and plant variety for the bruchid beetles. I focused on the female behaviour and fitness components (longevity, number of oviposition days, number of egg laid) as well as on several fitness components of their offspring (sex-ratio, development time, body size). But there are two other factors that are important in the life history of the bruchids that should be also considered : the density of beetles in the seeds which is determinant in terms of resource competition (e.g. Kawecki, 1995), and a "male effect" which is important in terms of genotype compatibility and ability to provide nutrients derived from their ejaculates, nutrients that females can use to increase their egg production, egg size and survivorship (Chen, 1984; Fox, 1993d; Fox *et al*., 1995; Eady *et al.*, 2000).

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Chapter 2

Behaviour and performance of two bruchid parasitoids on wild and cultivated beans : specialist vs generalist

Abstract:

In tri-trophic systems based on plants, the performance of a parasitoid is directly related to the quality of the host that it parasitizes, which in turn is a result of the host plant quality. When plant quality is altered, for instance through domestication, insects will have to adapt to these changes. Because specialists and generalists parasitoids are faced with different selection pressures during the process of host selection, they may respond differently to these plant changes. In beans from the genus *Phaseolus*, seeds from cultivated varieties contain higher levels of proteins and minerals and lower concentration and diversity of secondary compounds than their wild relatives

This study investigates the performance of a generalist and a specialist parasitoid that attack bean beetles on wild and cultivated seeds of *Phaseolus vulgaris*. Insects reared from field-collected wild *P. vulgaris* seeds were used to create 3 strains of each parasitoid species: 1. "wild" strain (W), reared on hosts feeding on wild seeds of *P. vulgaris*; 2. "cultivated" strain (C), reared on hosts feeding on cultivated seeds of *P. vulgaris* (Red Kidney variety) and 3. a "control" strain (N), reared on a third host species (*Vigna unguiculata*). After 12 generations in the laboratory, transplant experiments were conducted on the different seed types and the behaviour and performance of the parasitoids was followed.

I found significant differences in the behaviour and performance of both parasitoid species according to their origin of strain and the seed type. The specialist *Stenocorse bruchivora* is strongly influenced by the host quality and is selective when parasitizing a host. In contrast the most important factor in determining parasitism for the generalist, *Dinarmus basalis* appears to be host availability. These differences in behaviour are discussed in the context of the selective pressures that may operate on both parasitoid species.

Keywords: Parasitoid; *Stenocorse bruchivora*, *Dinarmus basalis*, domestication; performance; specialist; generalist; host suitability.

Introduction:

The successful development of a parasitic wasp is directly related to the quality of the host parasitized (review in Godfray, 1994). Size, age, and species of the host determine its nutrional quality (e.g. Pandey & Singh, 1999; Nicol & Mackauer, 1999; Neveu *et al.,*2000) and have an effect on the parasitoid fitness (e.g. Kenis, 1996; West *et al.,*1996; Ueno, 1999). But host acceptance does not imply host suitability for parasitoid growth and some hosts can produce lower quality parasitoids as for instance, smaller individuals (e.g. Rivers & Delinger, 1995; Godin & Boivin, 2000).

Host quality also results from the quality of the host plant on which the host feeds (Vinson & Barbosa, 1987). Several studies have demonstrated that plants have an influence on the development of the parasitoid directly or indirectly through the development of the phytophagous host (Fox *et al.,*1996; Turlings & Benrey, 1998). If the host develops on a non suitable host plant, then the development and fitness of a parasitoid are affected and not optimal (e.g. Kester & Barbosa, 1991). Plants may be non-suitable because they may lack nutritional compounds required for a successful development, and also because they may contain allelochemicals toxic to the insect. However, in some cases these chemicals can be used by the insects for their own defence. For example. more beetle larvae of *Oreina cacaliae* were parasitized by tachinid parasitoids, when they were feeding on plant (*Petasites paradoxus*) that do not contain pyrrolidizidine alkaloids (PAs). When their host plants contained PAs (*Adenostyles alliariae*, *A. glabra*), the beetle larvae stored and sequestered the PAs (Häggstrom H., 2000). The same was found with *Thelairia bryanti*, a tachinid fly that parasitize caterpillars of *Platyprepia virginalis* (Lepidoptera) (English-Loeb *et al*,. 1993). Whereas the proportion of parasitized *P. virginalis* is greater on poison hemlock (*Conium maculatus*) than on lupine (*Lupinus arboreus*), the caterpillars were more likely to survive parasitism and complete development (55 %) on hemlock than on lupine (40 %).

In the case of beans the domestication process has altered the amount and the type of allelochemicals present in the leaves and the seeds. Cultivated plants lost the ability to produce some secondary compounds found in the wild forms such as lectins, flavonoides, phenolic and cyanogenic compounds (Sotelo *et al.*, 1995; Linding *et al.,* 1997; Vanderborgth, 1979). Seeds from the cultivated forms have higher concentration of proteins and minerals than the wild seeds (Delgado *et al.*, 1999). Previous studies have shown that as resulted of domestication, cultivated plants provide a higher quality resource for both hosts and their parasitoids. Parasitoids that emerged from hosts reared on higher quality plant (cultivated) had a shorter development time and higher survival than parasitoids that emerged from hosts on suboptimal plants (wild) (Benrey *et al.,*1998). In addition,

these parasitoids were bigger and showed a higher fecundity. An explanation for an increase in insect performance on "good" hosts is explained by an increase in nutrients and a decrease in secondary compounds in cultivated plants (Benrey *et al.,*1998). Moreover, it has been suggested that larger parasitoids have a greater chance to mate and parasitize hosts (Godfray, 1994).

The influence that plant allelochemicals have on parasitic wasps mainly depends on the effects of those chemicals on their phytophagous hosts. These effects may vary according to the host specificity of the parasitoid. Barbosa (1988) reviewed the effects of nicotin concentration in host diet on the individuals parasitoid according to their polyphagy. As it was suggested for the *Cotesia* species (Brodeur *et al.,*1996), the acceptance phase of the hosts might be used as a reliable indicator of the parasitoid host specificity. Studies comparing specialist and generalist parasitic wasps are mainly focused on their foraging strategies and their use of chemical cues emitted by the host plant. Vet & Dicke (1992) hypothesized that the degree of specificity is linked to the specificity to identify these cues. The specialized parasitoids are likely to respond more to restricted stimuli, specific to their host species and it's immediate habitat, as opposed to the generalists which would be expected to respond primarily to higher level stimuli (e.g. habitat odour) (Waage 1979 in Vet & van Opzeeland, 1984). De Moraes & Lewis (1999) investigated the preferences of the specialist *Cardiochiles nigriceps* and the generalist *Microplistis croceipes* for the tobacco and cotton plant volatiles. *C. nigriceps* has a better ability to locate the hosts over long distances than *M. croceipes*, and displayed a superior host searching efficiency that may compensate for its disadvantage in intrinsic competition dominated by *M. croceipes* (De Moraes *et al.,*1999). But in comparison with the generalist *Cotesia marginiventris*, *M. croceipes* appears as a specialist, and did not show preference between artificially damaged cotton plants and undamaged control plants (Rose *et al.,*1998). Cortesero *et al*. (1997) showed that females of *C. marginiventris* were attracted by volatiles emitted from recent damage plants whereas females of *M. croceipes* preferred the host frass. The specialist *Leptopilina boulardi* responds strictly to the contact kairomone from its natural hosts (*Drosophila* associated to fruit substrate) whereas the generalist *L. heterotoma* responds to kairomones of a variety of *Drosophila* species associated to different decaying substrates (Vet *et al*. 1993). These last result supports the hypothesis that specialists require more specific information on the presence and suitability of their hosts than generalists (Vet & Dicke, 1992).

The foraging behavior can also be adapted to the host range specificity of the parasitoid: the generalist *Cotesia glomerata* expressed a relative plastic foraging behavior more adapted to search for gregarious hosts, whereas the specialist *C. rubecula* showed a fixed foraging behavior adapted to the solitary host such as, *Pieris rapae*

(Wiskerke & Vet, 1994). Moreover, Geervliet *et al.* (1996) showed no differences in odour recognition by these two parasitoids between plants infested with different caterpillar species or between plants infested with host and non-host species.

From the evidence presented above, it is clear that specialist and generalist use different cues to locate and accept their hosts. In addition, the suitability of the host is also an important aspect in the process of acceptance. Generalist and specialist parasitoids are faced with different selective pressures that may translate into differences in behavior when faced with the same type of host.

The aim of this study was to investigate the influence of host suitability mediated by the host plant and the behavior and performance of a specialist and a generalist parasitoid. The model system that I used includes wild and cultivated seeds of *Phaseolus vulgaris*, the bruchid *Zabrotes subfasciatus*, that attacks seeds on the genus *Phaseolus*, and two parasitoid species, the specialist *Stenocorse bruchivora* and the generalist *Dinarmus basalis*. Both species of parasitoids are commonly found in the field parasitizing hosts in wild and cultivated seeds. Some wild bean populations are isolated from each other while others grow side by side to cultivated field. Thus, I compared the performance and parasitism behavior of the specialist and the generalist parasitoid when exposed to bruchids reared on wild and cultivated beans that are known to vary in their nutritional quality and chemical composition. The hypothesis is that for both species, the hosts offered in cultivated seeds are a best resource for the development of the new generation, than the hosts offered in wild seed. I expect find a higher parasitism rate, a shorter develeopment time and bigger insects from the cultivated seeds of *P. vulgaris*.

In addition I investigated if parasitoids that are reared on hosts in only one type of seed (wild or cultivated) will perform better on these seeds. Both parasitoid species were reared on each seed type of bean (wild or cultivated) and also on another host plant species (*Vigna unguiculata* used as control), for several generations to obtain wasp strains. After this time, I conducted transplant experiments in which parasitoids from one strain were reciprocally tested on the various seed types (cultivated or wild). With these results, I expect first, that parasitoids originated from the "bad host quality" (the wild seeds) should perform better on wild seeds than parasitoids originated from the "better host quality" (cultivated seeds), and second, according to their host specificity, the generalist species *D. basalis* should be less sensitive to the host change than the specialist *S. bruchivora* (same larvae host species, but different host plant species).

Material & methods :

Zabrotes subfasciatus is one of the main pests of field crops and stored beans in Mexico and Central America. Females lay eggs which they glue on the seed coat. First instar larval burrow into the bean seed where they complete their development, and emerge as adults. *Stenocorse bruchivora* (Hymenoptera: Braconidae) and *Dinarmus basalis* (Hymenoptera: Pteromalidae) are two of the main parasitoids that attack this bruchid. They are both solitary ectoparasitoids that attack third and fourth instar larvae. Once a female parasitoid locates an infested seed, it will use a combination of chemical and mechanical cues (such as vibrations emanating from the seed) to locate the host (Perez & Bonet, 1984; Kumazaki *et al.*, 2000). Then she will introduce the ovipositor and will lay an egg on the outside of the beetle larva. *Stenocorse bruchivora* is originated and endemic of Mexico and Central America and it is considered as a specialist since it parasitizes only few host species (Hetz and Johnson, 1988). *Dinarmus basalis* originated from Africa, has a wider host range which has allowed it to colonize America, Asia and because of seed importation can sometimes be found in Great Britain and France (Rasplus, 1989).

Parasitoids were collected from infested seeds in several Mexican localities (Tepoztlan, Atila and Malinalco, see general introduction). For this experiment, the wasp populations used were chosen based on the

greatest number of parasitic wasps that emerged from the seeds. Thus, the 2 species of parasitoids originated from different sites. *Stenocorse bruchivora* was obtained from seeds collected in the Tepoztlan population, and *Dinarmus basalis* originated from seeds from the two other localities (Atila and Malinalco). Two sources of *Phaseolus vulgaris* seeds were used for this experiment. The wild seeds were collected in Tepoztlan (Mexico), the cultivated seeds, Red Kidney variety, and the cowpea seeds (Black eyes) were bought in a wholesaler of restaurant food. For each species (and each population of *D. basalis*) colonies were maintained for two generations on the bruchid *Zabrotes subfasciatus* offered in a common host different from the treatment seeds, *Vigna unguiculata* (cowpea "black eyes"). Each colony was divided into 3 groups : the first one ("wild strain" **W**) was reared on hosts offered in wild seeds of *Phaseolus vulgaris* seeds, the second one ("cultivated strain" **C**) on hosts offered in cultivated *Phaseolus vulgaris* seeds (Red Kidney variety) and the third and last group ("control strain" **N**) on cowpea (*Vigna unguiculata*).

Experimental design:

For each new wasp generation, I offered unparasitized hosts on the same seed type from which the female wasps had originated. The hosts offered in cowpea and Red Kidney were 18 days-old; those offered in the wild seeds were 21 days-old. These differences in the age of hosts offered was to account for differences in development time. After several generations (11 for *Stenocorse* and 12 for *Dinarmus*). I conducted transplant experiments, in which parasitoids from each strain (W, C N) were reciprocally tested on the various seed types (c or w).

To test the effect of the seed type on which parasitoids were reared on the fitness components, I allowed each female wasp to parasitize hosts offered in both types of seed (wild versus cultivated). Each newly emerged virgin female wasp (G0) was placed in a gelatine capsule with a virgin male originated from the same batch during 24 h. Subsequently I placed all couples together in a plastic box with honey (to make sure that females would mate). After 4 days I placed one couple of wasps in a plastic box with seeds infested by a total of 15 *Zabrotes* larvae of 19 or 20 days olds. Because in the past we have found that when a female is alone she will be hesitant to parasitize, the box was covered by a piece of nylon and placed in an aquarium with several inexperienced wasps. This step was to stimulate females to parasitize the seeds by having several females in the same cage. I allowed the females to parasitize for a period of three days. Because the sex ratio of the progeny is dependant of the age of the female (Kenis, 1996), all the females were tested when they were fourth days old.

For the test with wild seeds, I used 15 seeds infested each, by a single *Zabrotes* larva of 20 days old. For the cultivated seeds, I offered the 15 *Zabrotes* larvae in the following pattern: 1 larva in the first bean seed, 2 larvae in the second bean, 3 larvae in the third bean, 4 in the next one and 5 larvae in the last bean. This allowed to control for host density and host distribution in all replicates.

Each newly emerged wasp (G1) was recorded at its emergence and placed in alcohol. At the end of the experiment a dissection of all the seeds was done to identify hosts or wasps that had died inside the seed. I recorded the number of wasps that emerged from each seed, their development time (days between the third experimental day and the adult emergence) the sex and the length. Sex ratio is the number of parasitoid males divided by the number of total parasitoids that emerged. I also recorded a parameter that I called "motivation" which refers to the proportion of G0 females that parasitized the offered seeds, which is different from the "parasitism rate" calculated only with females that parasitized the seeds, and which is the number of newly emerged parasitoids divided by the total number of offered hosts.

Statistical analyses:

Because data did not meet model assumptions of normality and homogeneity of variance, I used non-parametric tests (Sokal & Rohlf, 1995). I analyzed the proportion of females that parasitized hosts offered in seeds (motivation) using contingency tables and χ^2 test. To investigate the effect of seed type and wasp origin on sex-ratio, parasitism rate, larval development time and adult size, Kruskal-Wallis tests were performed.

When significant differences were found among treatments, we completed the analysis with a Wilcoxon Rank test with a significant level (α') corrected according to the number of comparisons for each group, using the Dunn-Sidak adjustment (Sokal and Rohlf, 1995) : $\alpha' = 1- (1 - \alpha)^{1/k}$ with $\alpha = 5\%$ and k = number of comparisons. The treatments tested were Cc, Cw, Wc, Ww, Nc, and Nw where C, W and N refers to the origin of the wasps and c, w, refer to the seed type tested. For example Cc is first compared to Cw (to test for the seed effects) than to Wc and Nc (to test for wasp strain effects), k = 3 and $\alpha' = 0.016$.

Effect of the wasp strain: if a significant effect of the seed type was found, the comparison between strains was done within each type of seed using the Wilcoxon Rank test. When no significant effect of seed type was found, the wasp strain effect was analyzed with a Kruskall-Wallis test, pooling together the results of the strain on both wild and cultivated seeds.

Effect of the seed type: if a significant effect of the wasp strain was found, the influence of the seed type was tested between each strain using the Wilcoxon Rank test. When no significant effect of the strains was found, the influence of the seed type used during the test was determined with a Mann-Whitney U test, pooling together the results obtained for the different wasp strains.

I used the S-Plus 2000 statistical program to perform the tests (S-PLUS 2000).

Results:

Names of the treatments are indicated with first the letter of the wasp strain (**C**, **W** or **N**) followed by the letter of the seed type used for the test (**c** or **w**). For example, **Cw** refers to wasps from the cultivated strain(**C**) tested on wild seeds(**w**), and **Nc** refers to the wasps from the control group (**N**: cowpea) tested on cultivate seeds (**c**). For each parameter and for each treatment, the sample size tested is in Table 1.

Specialist versus generalist:

I found clear differences in the behavior and performance of the specialist *S. bruchivora* and the generalist *D. basalis*. *S. bruchivora* was more selective on the same species of plant, type of seed and the host quality, whereas *D. basalis* showed a more active parasitism behavior on all available hosts, which lead to variation in the offspring performance. These findings are based on the results on the interaction between the seed type and the origin of the wasp. I develop these results in the section below.

Motivation: (proportion of females that parasitized the seeds)

S. bruchivora (the specialist): I found significant differences on the proportion of females that parasitized the seeds among the 3 group of the wasps (cultivated, wild, and control strains). (Fig. 1a). Wasps originating from *P. vulgaris* seeds (C and W) were more willing to parasitize beans seeds than wasps originating from cowpea (χ^2 = 19.11; df = 2; $p < 0.0001$). There was no significant difference between the wild and the cultivated strains (χ^2 =

2.50; df = 1; p = 0.11). Also significant differences were found between the seed type used (cultivated versus wild). More females parasitized when hosts were offered in cultivated seeds than in wild seeds ($\chi^2 = 31.16$; df = 1; $p < 0.0001$).

D. basalis **(the generalist): For parasitoid of both populations, Atila and Malinalco, the wasp origin and the seed type used did not affect the proportion of females that parasitized the hosts. Almost all females parasitized the hosts offered (Atila: wasp origin: $\chi^2 = 0.3$; df = 2; p = 0.86; seed type: $\chi^2 = 1.33$; df = 1; p = 0.248; Malinalco: wasp origin: $\chi^2 = 3.13$; df = 2; p = 0.21; seed type: $\chi^2 = 0.73$; df = 1; p = 0.39) (Fig. 1b; Fig. 1c).**

	Stenocorse bruchivora							*Dinarmus basalis*														
								Atila							**Malinalco**							
	Motivation (couples)	Parasitism rate	Sex-ratio	Development time male	Development time female	Tibia size male	Tibia size female	Motivation (couples)	Parasitism rate	Sex-ratio	Development time male	Development time female	Tibia size male	Tibia size female	Motivation (couples)	Parasitism rate	Sex-ratio	Development time male	Development time female	Tibia size male	Tibia size female
Cc	37	25	25	17	30	15	31	24	22	22	59	86	45	85	23	23	23	65	97	58	98
Wc	37	29	29	29	44	30	49	23	21	21	64	61	52	67	24	24	24	76	79	61	96
Nc	37	14	14	17	11	14	11	20	20	20	56	74	59	70	24	22	22	62	80	48	92
Cw	43	11	11	11	5	11	5	24	21	21	41	21	34	25	23	21	21	37	24	27	28
Ww	43	17	17	8	14	7	14	23	21	21	36	17	24	21	24	23	23	43	24	34	26
Nw	43	5	5	7	2	6	6	22	19	19	32	25	24	27	23	21	21	41	36	34	40

Table 4: Sample size (n : number of individuals) used for the analysis of the effect of wasp strain (C, W, N) and seed type (c, w) on the behavior and performance of *S. bruchivora* and individuals of two populations of *D. basalis* (Atila and Malinalco)

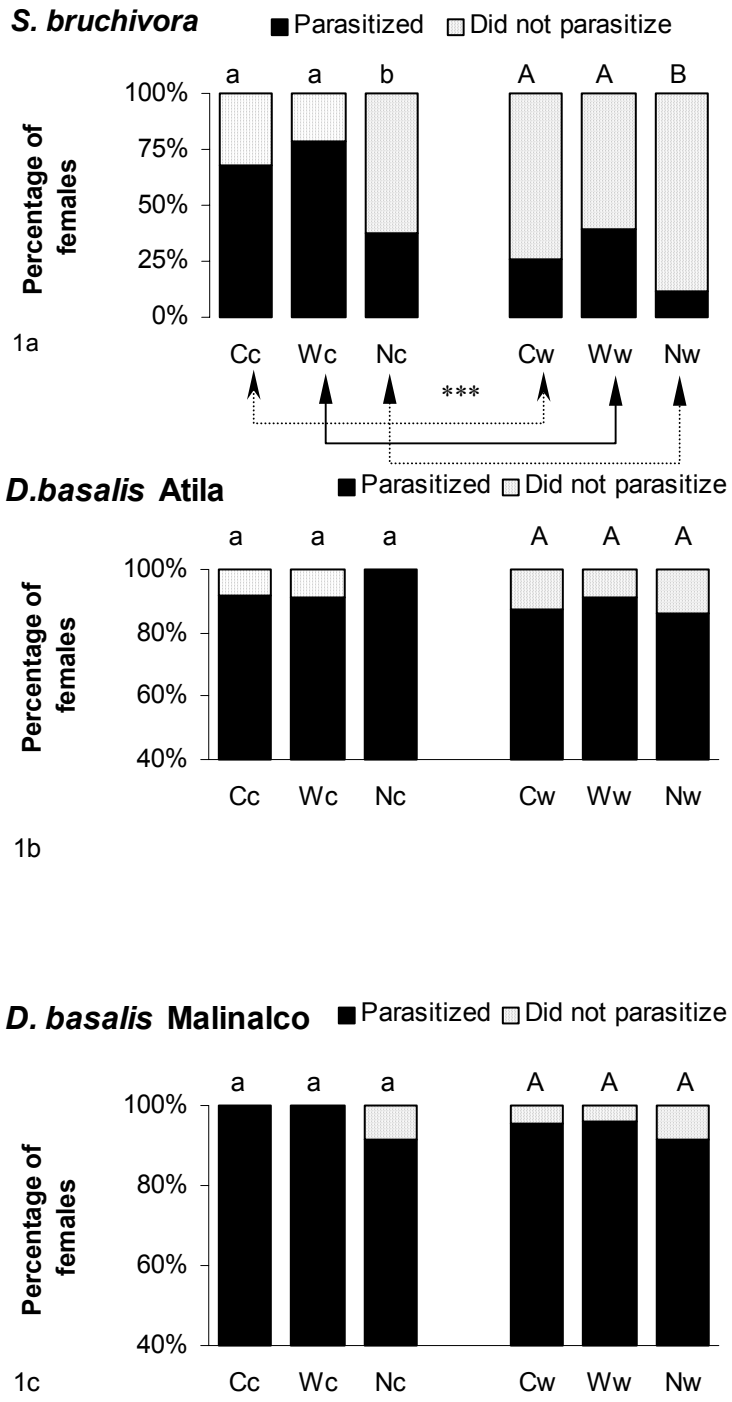
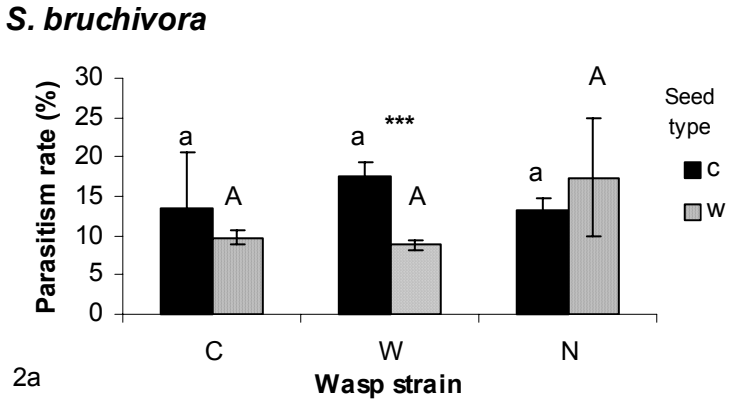


Figure 2: Percentage of females of three strains (C: cultivated, W: wild, N: control) that parasitized hosts in wild (w) and cultivated (c) seeds. 1a: *S. bruchivora* ($37 < n < 43$); 1b: *D. basalis*, Atila ($20 < n < 24$); 1c: *D. basalis*, Malinalco ($23 < n < 24$). The arrows and the asterisks indicate significant differences for one strain due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains.

Parasitism rate:

S. bruchivora (the specialist): The seed type had an influence only for the "wild strain"(Fig. 2a). Wasps from this group (Ww) parasitized fewer hosts on the wild seeds than on the cultivated seeds ($Z = 3.303$; $p = 0.001$). But this low parasitism rate is not significantly different from the parasitism rate of the Cw group ($Z = 0.82$; $p = 0.41$) (wasps that originated from the cultivated strain tested on wild seeds). It may be that even though the wild strain originated from wild seeds, once they are exposed to hosts on cultivated seeds, they tend to parasitize these more because they offer a better quality host. There is no strain influence inside each seed group ($0.11 < p < 0.86$).

D. basalis (the generalist): For both populations and each strain, hosts offered in cultivated seeds were more parasitized than hosts offered in wild seeds (Atila: $p < 0.001$; Malinalco: $p < 0.0001$) (Fig. 2b & 2c) which means that hosts offered in the cultivated seeds represented a better host quality or were easier to parasitize. There was no effect of the wasp origin within each seed group (Atila : $0.25 < p < 0.9$; Malinalco: $0.11 < p < 0.89$). The parasitism rate by the two populations of *D. basalis* is clearly higher than by *S. bruchivora*, doubled on wild seeds and around three time more on cultivated seeds. Although these results don't allow to separate species effect from site effect (1 species, 1 population), personal observations with other populations of these parasitoids allow me to assume that the "species" is the origin of such difference in the parasitism rate.



2a

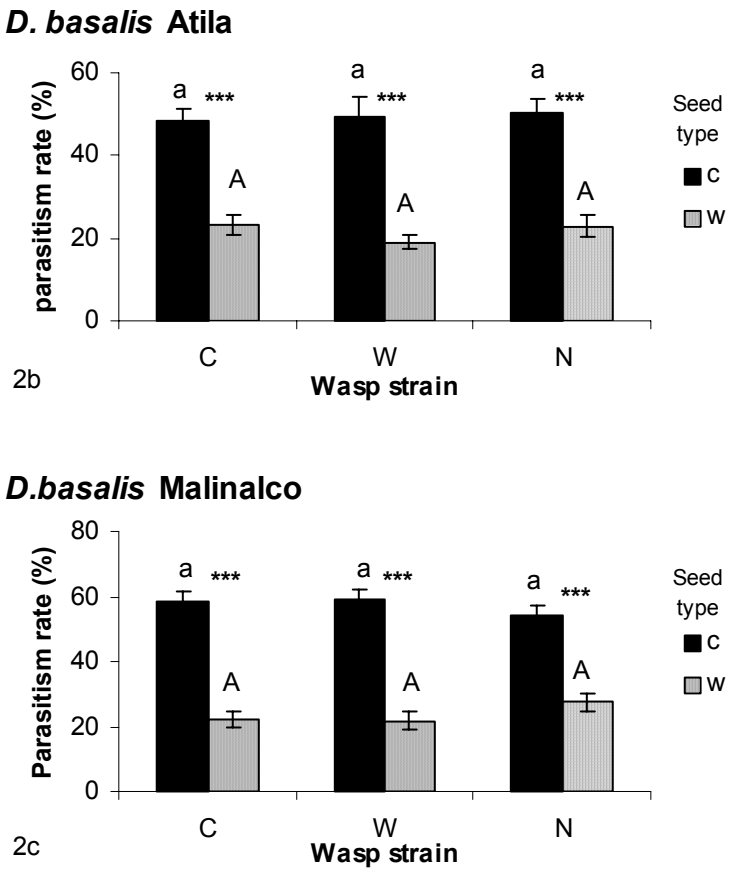


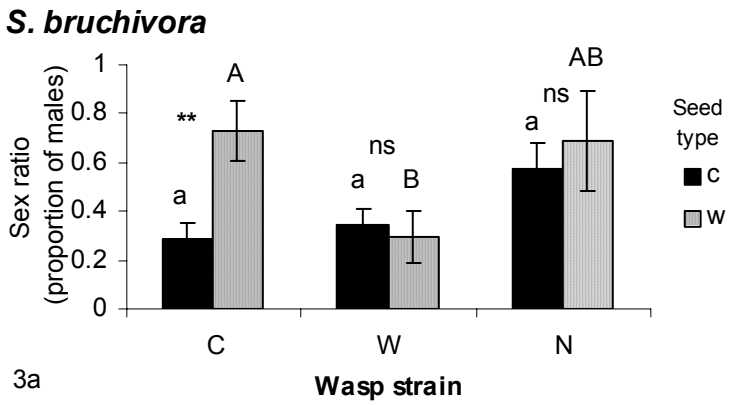
Figure 3: Parasitism rate (as the number of parasitized hosts by each female), according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 2a: *S. bruchivora* (5 < n < 29); 2b: *D. basalis*, Atila (19 < n < 22); 2c: *D. basalis*, Malinalco (21 < n < 24). Asterisks indicate significant differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains on wild seeds (a,b) or on cultivated seeds (A, B).

Sex-ratio:

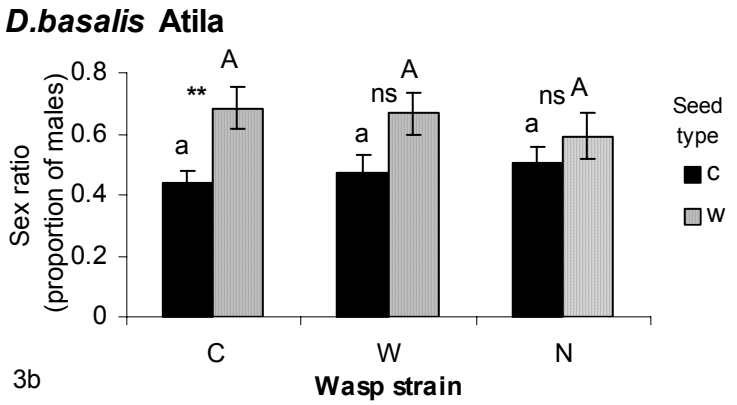
S. bruchivora (the specialist) (Fig. 3a): I found a significant difference among the treatments ($H = 14.758$; $p = 0.011$) due to the seed type and the strain. It seems that the type of the seed has an effect for the cultivated wasp strain only. The sex ratio is significantly biased towards females (0.28) on cultivated seeds (Cc), and towards males (0.73) on wild seeds (Cw) ($Z = -2.77$; $p = 0.005$). There was no effect of the seed type for the other two strains (control and wild strain) (Nc vs Nw: $Z = -0.53$; $p = 0.59$; Wc vs Ww: $Z = 0.91$; $p = 0.36$). The strain influence was found on the wild seeds: the sex ratio of the Ww group (0.29) is biased toward females and different from the sex ratio of the Cw group (0.73) ($Z = 2.36$; $p = 0.018$) which suggests that hosts offered in

wild seeds are suitable host for the females of the wild strain. This sex ratio was not statistically different from the sex ratio of the Nw group ($Z = -1.61$; $p = 0.11$), but this can be due to the low effective ($n = 5$).

D. basalis (the generalist): For the population of Atila (Fig. 3b), a significant difference due to the seed type was found only for the cultivated strain ($0.0001 < p$), whereas the wild and cultivated strains of the population of Malinalco (Fig. 3c) showed a sex ratio significantly biased toward the females on cultivated seeds, and male biased on wild seeds (Wc vs Ww: $p = 0.0018$; Cc vs Cw: $p = 0.0084$). For both populations the sex ratio of the control strain did not differ significantly between wild and cultivated seeds ($0.013 < p < 0.21$). No significant strain effect was found on the sex ratio, for both populations on wild or cultivated seeds ($0.21 < p < 0.81$).



3a



3b

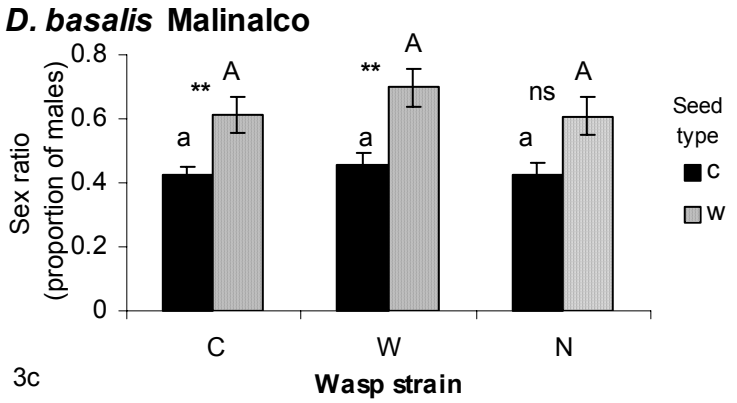


Figure 4: Secondary sex ratio expressed as the proportion of males, according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 3a: *S. bruchivora* (5 < n < 29); 3b: *D. basalis*, Atila (19 < n < 22); 3c: *D. basalis*, Malinalco (21 < n < 24). Asterisks indicate significant differences due to the seed type: * p < 005; ** p < 0.01; *** p < 0.001. Different letters indicate significant differences between the strains on wild seeds (a,b) or on cultivated seeds (A, B).

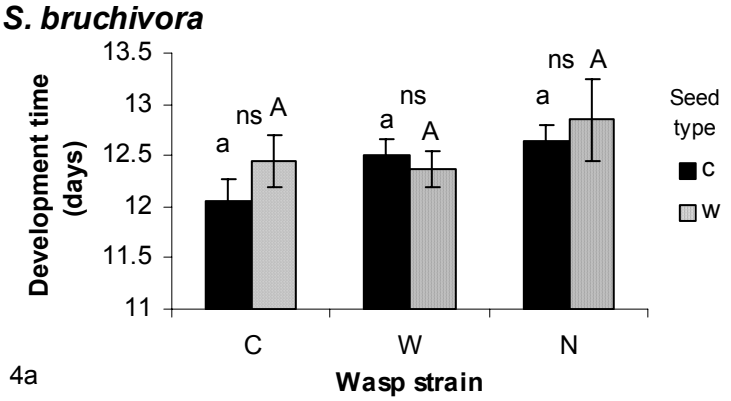
Development time:

S. bruchivora (the specialist): For both sexes, the larval development time was not influenced by the seed type used or the wasp strain (male: seed type: Z = - 0.46; p = 0.65; wasp strain: H = 4.54; p = 0.10; female: seed type: Z = - 0.02; p = 0.98; wasp strain: H = 0.95; p = 0.62) (males: Fig. 4a; females: Fig. 5a). Males take between 12.06 +/- 0.2 (Cc) to 12.86 +/- 0.4 (Nw) days before their emergence. Females take on average 1 day more to achieve their development : 13.0 +/- 0 (Nw) or 13.57 +/- 0.29 (Ww).

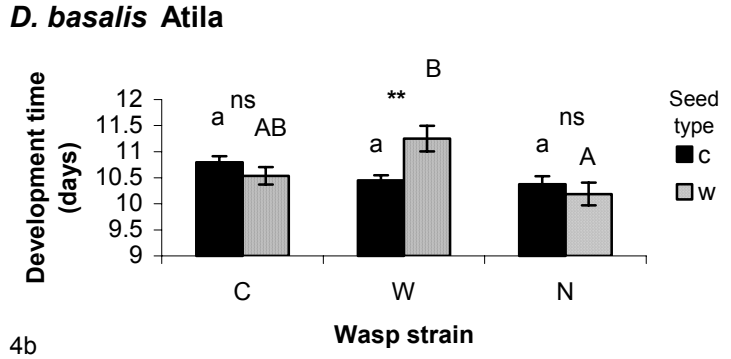
D. basalis (the generalist):

Atila: There was an effect of the seed type on the males originated from the wild strain (Fig. 4b): they emerged earlier when they came from cultivated seeds (p = 0.005). There were no differences between the strains within each seed group (0.018 < p < 0.44) except on wild seeds where males that originated from the control strain emerged earlier than males that originated from the wild strain (p = 0.0013). For the females, there was no effect of the seed type (0.025 < p < 0.61) (Fig. 5b). This allowed the comparison of the strains without seed distinction, which revealed that there was an effect of the wasp origin (H = 12.98; p = 0.0015). Females that originated from the control strain emerged earlier than females that originated from the cultivated strain (Z = - 3.48; p = 0.0005).

Malinalco: There were no differences between the treatments for the two sexes (Males: H = 8.09, p = 0.15; Females H = 10.08, p = 0.073). I pooled the data from the different treatments to test the effect of the strains and of the seed type on the larval development time. There was no effect of the wasp strain on the development time of both sexes (males: H = 1.77; p = 0.41; females: H = 2.13; p = 0.34), but there were differences due to the seed type used. For both sexes, wasp originating from the wild seeds develop faster



4a



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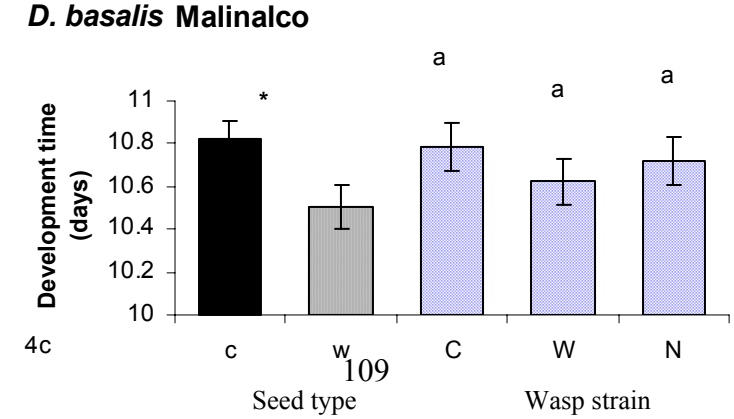
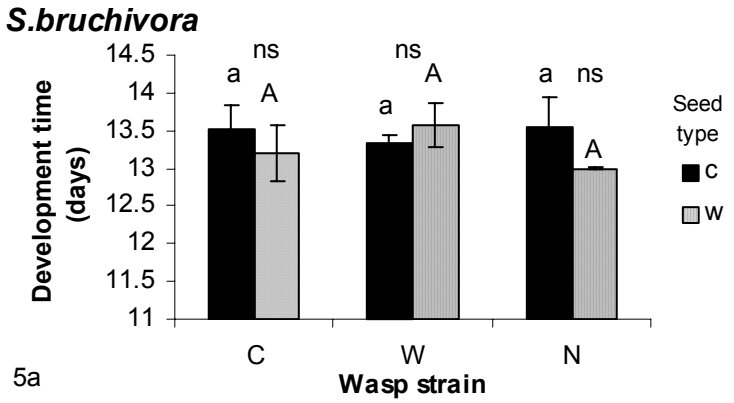
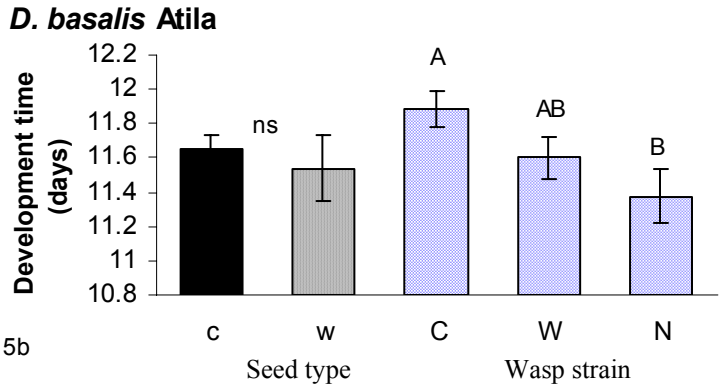


4c

Figure 5: Larval development time of the male offspring, according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 4a: *S. bruchivora* (7 < n < 29); 4b: *D. basalis*, Atila (32 < n < 64); 4c: *D. basalis*, Malinalco (37 < n < 76) where data were pooled according the wasp strain or the seed type. Asterisks indicate significant differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains on wild seeds (a,b) or on cultivated seeds (A, B).



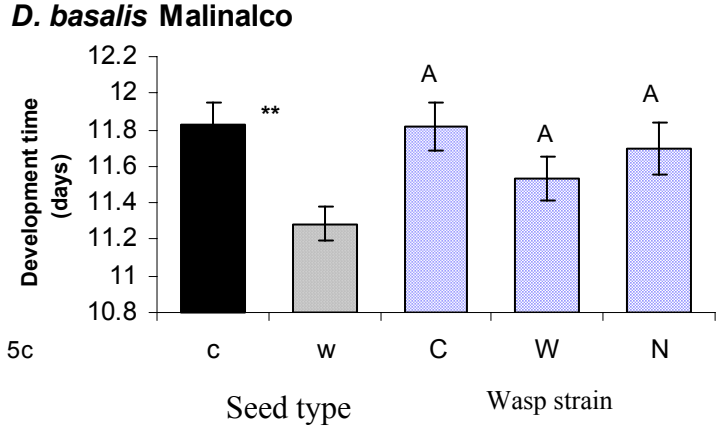
5a



5b

Figure 6: Larval development time of the female offspring, according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 5a: *S. bruchivora* (2 < n < 44); 5b: *D. basalis*, Atila (17 < n < 86) were data were pooled according the wasp strain or the seed type; 5c: *D. basalis*, Malinalco (24 < n < 97) where data were pooled according the wasp strain or the seed type. Asterisks indicate significant differences due to the seed type: * p < 0.05; ** p < 0.01; *** p < 0.001. Different letters indicate significant differences between the strains on wild seeds (alb) or on cultivated seeds (A, B).

than the wasps originated from the cultivated seeds (males: Z = -2.13; p = 0.03; females: Z = -2.77; p = 0.0057) (males: Fig. 4c; females: Fig. 5c).



Size:

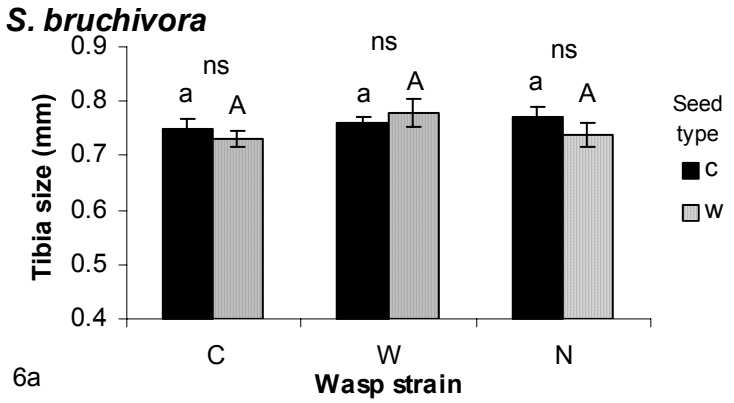
S. bruchivora (the specialist): For both sexes, there is no difference between treatments in the tibia size (male: H = 5.03, p = 0.41; female: H = 11.26, p = 0.046). The seed type and the wasp strain had no effect on the tibia length of the parasitoid. The average size of male tibia is between 0.73 +/- 0.014 mm (Cw) to 0.78 +/- 0.026 mm (Ww). For the females, tibia size is between 0.67 +/- 0.02 (Nw) to 0.71 +/- 0.01 (Cw) (males: Fig. 6a; females: Fig. 7a).

D. basalis (the generalist):

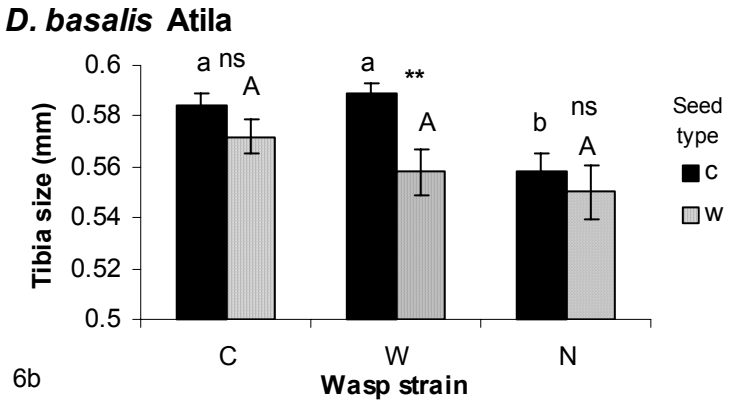
Atila: A significant difference was found between males of the wild strain due to the seed type (Fig. 6b): males that emerged from the cultivated seeds are larger than males that originated from the wild seeds (p = 0.0029). On cultivated seeds, males from the control strain were significantly smaller than in the two other strains (Wc vs Nc: p = 0.0008; Cc vs Nc : p = 0.0045). For the females (Fig. 7b), there were no differences in their size due to the seed type inside by strain, or differences between the strains within each group. Data were

pooled to test the effects of seed type and it was found that females that emerged from wild seeds are smaller than females that emerged from cultivated seeds (Z = - 2.67; p = 0.007). There was no effect of the wasp strains on the tibia size of the females from Atila (H = 4.59; p = 0.10).

Malinalco: For all the strains there was a strong effect of the seed type on male tibia size (Fig. 6c): males that originated from wild seeds are smaller than males that originated



6a



6b

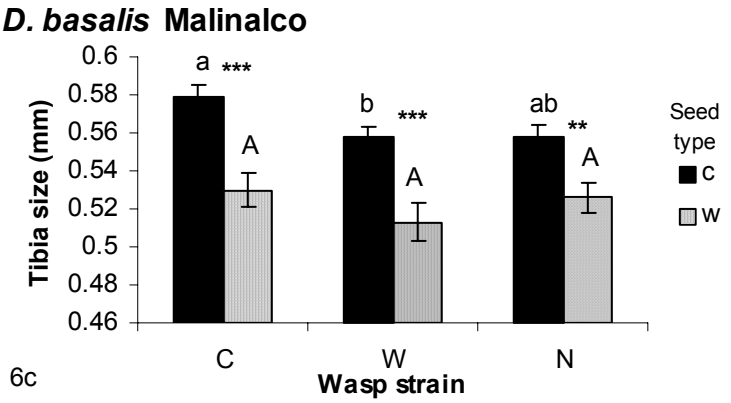
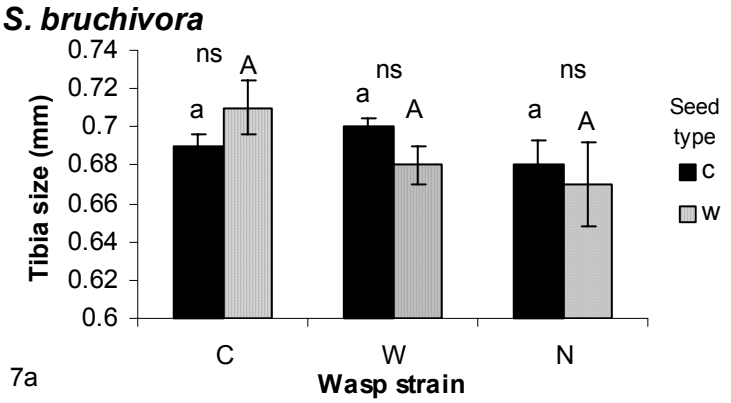
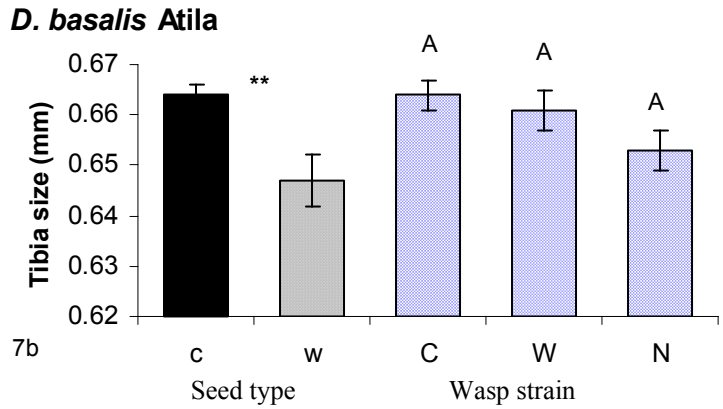


Figure 7: Tibia size of the male offspring, according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 6a: *S. bruchivora* (6 < n < 30); 6b: *D. basalis*, Atila (24 < n < 59); 6c: *D. basalis*, Malinalco (27 < n < 61). Asterisks indicate significant differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains on wild seeds (a,b) or on cultivated seeds (A, B).





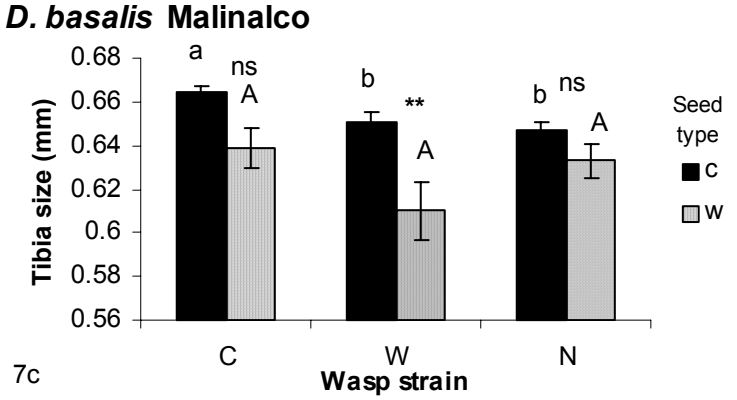


Figure 8: Tibia size of the female offspring, according to the strain (C: cultivated, W: wild, N: control) and the seed type (c: cultivated, w: wild). 7a: *S. bruchivora* (5 < n < 49); 7b: *D. basalis*, Atila (21 < n < 85) where data were pooled according the wasp strain or the seed type; 7c: *D. basalis*, Malinalco (26 < n < 98). Asterisks indicate significant differences due to the seed type: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Different letters indicate significant differences between the strains on wild seeds (a,b) or on cultivated seeds (A, B).

from the cultivated seeds ($0.0002 < p < 0.0098$). Moreover, on cultivated seeds, males from the cultivated strain are significantly larger than males from the wild strain ($p = 0.0098$) and close to be larger than male from the control strain ($p = 0.018 > 0.016 = \alpha$). For the females, a seed effect was found only on the wild strain : females that emerged from the cultivated seeds are larger than females that emerged from wild seeds ($p = 0.004$) (Fig. 7c). On the cultivated seeds, females from the cultivated strain are larger than females from the two other strains ($0.008 < p < 0.014$).

Discussion:

The results found in this study show that both the strain of origin of the parasitoid and the seed type, influenced the behaviour and fitness of the parasitic wasps. But these results also showed that there is great variation between species and between populations within the same species. In the light of personal observation on parasitism rate and behaviour, differences between *S. bruchivora* (the specialist parasitoid) and *D. basalis* (the generalist parasitoid) are clear in this experiment, whereas the differences found between populations for the generalist *D. basalis* are not obvious. The first hypothesis that cultivated seeds represent a better resource than the wild seeds for the parasitic wasps, is an evidence for *D. basalis* according the populations, whereas *S. bruchivora* confirms my hypothesis of a possible adaptation by the wasps to a bad host seed quality after several generations. The last hypothesis, the difference in "fidelity" between the two wasp species on the host plant species is also confirmed by my experiments, with a major impact of the host plant change for the specialist parasitoid in its behaviour than for the generalist. Overall it was found that the specialist *S. bruchivora* is more selective. Its parasitism behaviour is influenced by its seed of origin and the host quality on these seeds, whereas the females of the generalist *D. basalis* parasitized all available hosts and because of this, showed a greater variation in the performance of their offspring.

According to their original seed type, females of *S. bruchivora* that originated from *Phaseolus vulgaris* (both wild and cultivated seeds) were more willing to parasitize the seeds, and more females parasitized when hosts were offered in cultivated seeds. For *D. basalis*, neither the strain of origin nor the seed type influenced the behaviour of the females. Females of *D. basalis* have are more likely to parasitize any available hosts

which confirms the greater plasticity in the parasitism behaviour of *D. basalis* versus *S. bruchivora*

Contrary to the motivation to parasitize, the parasitism rate expressed by the females *S. bruchivora* that parasitized the hosts, is usually not dependent of the strain or the seed type except in the case of the wild strain (W). There was a higher parasitism rate by this strain on cultivated seeds. This is consistent with the fact that hosts from cultivated seed are better for the development of the wasps (Callejas, 1996). It is likely that females from the wild strain are more sensitive to the host quality because they come from bad quality hosts (on wild seeds) and, hosts offered in cultivated seeds represent a clearly better host quality. The greater parasitism rate of the generalist *D. basalis* on the cultivated beans suggests that larvae are easier to be found in these seeds than on wild seeds. Perhaps this can be due to the larger host size of the host in the cultivated seeds, or to the fact that in some seeds, there are up to 5 larvae available compared to only one larva in the wild seed. However, this pattern was not found for *S. bruchivora*. This can be consistent with our findings on the two different parasitization strategies of these two species, according to their host specificity. The females of the generalist *D. basalis* tend to parasitize as many hosts as possible. Their main constraint is to locate the host, and in this case a higher host density (up to 5 larvae in one single cultivated seed) increases their parasitism rate on cultivated seeds. In contrast, the females of the specialist *S. bruchivora*, are more concerned with the quality of the host. Therefore their main constraint is the suitability of the host. Whatever the host density is, the females allocate time to evaluate the suitability of the host. Thus, for this species, the parasitism rate is not influenced by the host density. Moreover, in this experiment the larval density was optimal for the development of the wasp, with one single larva on wild seeds, and up to 5 larvae in the cultivated seeds. It has been shown that up to 10

larvae can successfully complete their development in cultivated seeds (Callejas, 1996; personal observation).

Rios (1998) investigated the performance and behaviour of *S. bruchivora* on wild and cultivated seeds of *Phaseolus coccineus* seeds, and showed that cultivated seeds offer the best resource for the parasitoid. Females of *S. bruchivora* were more attracted and stayed more time in contact with the cultivated seeds *of Phaseolus coccineus (P. c. darwininianus* and *P. c. coccineus*) than on the wild seeds of *P. c. formosus*. But this attraction was not due to the volatiles emitted by the seeds. Also, in choice experiments the females parasitized more hosts in the cultivated seeds than the wild seeds (27.7 to 32.8 % versus 9.7 %). These low parasitism rates were obtained after 7 days of parasitization which confirms that in the laboratory, females of *S. bruchivora* are very hesitant to parasitize hosts in wild seeds.

Host quality did not influence the larval development time and adult size of *S. bruchivora*. These results are opposite to those obtained by Rios (1998). In some of her experiment, she found a significant effect of the seed type (and seed quality) on the parasitism rate, the sex ratio, the development time and the size of the offspring. The differences between these two experiments could be due to the different host plant species used (*P. coccineus* versus *P. vulgaris*) that may affect differently the development of the host which in turn significantly affect the fitness parameters of the parasitoid.

For *D. basalis*, both the seed type and the wasp strain influenced the development time and the adult size. The wasp strain mainly influenced the development time in the Atila population, whereas it was the seed type that influenced it in the Malinalco population. In Atila, females development time was not different due to the seed type, whereas in Malinalco, wasps from cultivated seeds, developed slower than females from

the wild seed. These results are not in accordance with the prediction that development is faster on higher quality host (Benrey *et al.,*1998). However, wasps from the cultivated strain or from the cultivated seeds tend to be larger than those from the wild seeds or the other strains which does support the higher host quality in cultivate seeds. Better quality host will result in larger offspring, but necessitate longer development time to consume all the host.

Several environmental and genetic factors may influence the offspring sex-ratio such as, the temperature, the photoperiod, the relative humidity, the host size, age and quality, the female wasp density, their maternal age, size and diet, (review in King, 1987). By using females of the same age and rearing experience, I tried to control for most of these. For this experiment we can reasonably exclude a differential mortality effect between the sexes. If differential mortality was the responsible factor for the results observed, I would expect to find a low insect emergence (both bruchids and parasitoids). However, from the 15 hosts offered, almost of the non parasitized hosts emerged (personal observation). Therefore, there is strong evidence that the secondary sex ratio analysed is the one allocated by the female parasitoid during the oviposition. The theory of the sex allocation theory (Charnov *et al.,*1981; Charnov, 1982) predicts that a female biased sex-ratio should occur on larger and better quality hosts. This theory has been supported in studies with several parasitoids species, among these: *Metaphycus flavus*, *Coeloides sordidator*, and *Pteromalus cerealeillae.* Larger hosts led to more female offspring (Bernal *et al.,*1999; Kenis, 1996; Wen *et al.,*1995). Similarly, the females of the aphid parasitoid *Lysiphlebia mirzai* adjust their progeny sex ratio according to the host: they deposit fertilized eggs in large hosts (third instar nymphs) and unfertilised eggs in small hosts (first and second instars nymphs). But the perception

of the host size is dependent on their previous parasitization experience (Pandey & Singh, 1999). For *Trybliographa rapae*, hosts of the third instar favored the oviposition (with a faster rate of development and a larger body size of the offspring) but the sex-ratio of the progeny was not different from that in other larval instars (Neveu *et al.*,2000).

Our results on the sex-ratio are not consistent with the sex allocation theory for the specialist *S. bruchivora*, except with the cultivated strain. For this same species, Rios (1998) also found a biased sex ratio towards males on cultivated bean seeds of *Phaseolus coccineus*. In my experiment, the sex ratio was not only influenced by the seed type (which result in a better or worst host quality), but was also affected by the strain of the wasp. In the case of the cultivated strain we can explain the influence of the seed by an effect due to the switch of hosts from cultivated to wild seeds, the second one being of poorer quality resulting in a male biased sex-ratio. But for the females of the wild strain , these "poor" quality hosts are the hosts from which they are originated and used as a reference. Therefore they "estimate" this type of host as good enough for the development of female offspring.

In contrast, the results on the sex ratio for the generalist *D. basalis* do support the sex allocation theory. The sex ratio is female biased on cultivated seeds, and more male biased on wild seeds whatever the origin of the female is. A female-biased sex ratio on better quality hosts by *D. basalis* was also found by Fujii & Wai (1990). Females allocated more females offspring in older (and larger) hosts.

This experiment provided also evidence for a potential effect of the rearing of the two parasitoid species on the different type of seeds. For the specialist *S. bruchivora*, the fact that the sex-ratio of the Ww group is significantly more female biased than the sex-ratio of the Cw group, could be the result of adaptation to lower quality hosts in these seeds. Indeed, the females of the wild strain are adapted to develop in a "bad" host which is sufficient for the development of diploid females. With the generalist *D. basalis*, this bias in the sex ratio for

the wild strain tested on wild seeds was not observed for the two populations. It seems that the rearing on the poor host during 12 generations did not affect their perception of the quality of the host offered.

The differences found between the strains, are relevant because when beans are not available, parasitoids may need to use alternative hosts and/or host plants to survive. *S. bruchivora* is strongly influenced by its original host plant in its parasitism behaviour (the number of wasps that parasitize the beans is lower when females originated from the cowpea seeds) whereas it is not the case for *D. basalis*. The specialist *S. bruchivora* is found in the New World where it can parasitize several host species (Hetz & Johnson, 1988) whereas *D. basalis* parasitizes also hosts on several plant species and has a much wider distribution. It is found in Africa, America and Asia (Rasplus, 1989). This may explain its greater ability to parasitize easily on several host plants even, novel ones. The greater plasticity in parasitism behaviour may lead to a higher variability in behaviour and parasitism strategies which can explain the variability found between our populations. A comparison among several populations of *S. bruchivora* would allow a better understanding of the importance of the original host plant on the parasitism behaviour of this species.

Another interesting point is the response of the control strain. In the case of *Stenocorse bruchivora*, wasps that originated from cowpea (*Vigna unguiculata*) were not willing to parasitize hosts in the *Phaseolus vulgaris* seeds, which was not the case for *D. basalis*. This result could be associated to the adaptability of the generalist parasitoids to respond to several non specific cues, e.g. leaf volatile compounds even in undamaged plants such as, for the parasitoid *Glyptapanteles flavicoxis* on its host plant *Populus nigra*, (Havill & Raffa, 2000). Whereas a specialist is able to make the distinction among hosts and non host species such as, *Microplitis croceipes* that can discriminate among volatiles emitted from frass of several host species (Rose *et al*., 1997). In my study, we could hypothesize that the specialist *S. bruchivora,* responded preferentially to specific compounds emitted by bean seeds and not present in cowpea seeds, whereas the generalist *D. basalis* responded to general cues emitted by both plant species, or by the volatiles emitted from the host frass, that was identical during the rearing period and the test. In my transplant experiment from *V. unguicularis* seeds to *P. vulgaris* seeds, the volatiles emitted by the seeds changed, whereas the volatiles emitted by the frass of the host did not changed: the same host species was used. In the case of *D. basalis*, due to pre-emergence learning, females placed as pupae in capsules together with host plant fragments or with herbivore host larval remains became

sensitive to these odours (Monge & Cortesero, 1996). Until now, no investigation was done on *S. bruchivora* learning ability.

It is often considered that generalist species, because they are found in more variable environments, have a greater learning ability than specialist species. Thus, the ability to learn to use host-related cues might be associated to polyphagy (Vet & van Opzeeland, 1984). It is more likely that if there was any pre-emergence learning in *S. bruchivora* would be associated to the host plant odour and not to the bruchid host. Females that originated from cowpea seeds, did not recognize the bean seeds as their natural host plant, and were not interested in investigating the bruchid host inside this novel host plant. But when the females investigated the presence of infested seeds and the host quality, this resulted in the same parasitism rate of the bruchid host. Also, the females from the different strains, preferred to parasitize when hosts were offered inside the cultivated seed, which is in agreement with the better host quality. For *D. basalis* most of the tested females decided to parasitize the offered hosts independent of the seed type offered. Perhaps this generalist parasitism is due to the pre-emerging learning based on the host odour (Monge & Cortesero, 1996) which is more efficient than the possible learning mechanism of *S. bruchivora* which relies more on the host plant and not on the bruchid host.

***Stenocorse bruchivora* and *Dinarmus basalis* were reared under the exact same conditions. This allowed me to estimate the potential of each species to compete with each other. Of all the females tested, the maximum number of *S. bruchivora* females that parasitized the seeds was around 78 % on cultivated seeds, and 39.5 % on wild seeds. Whereas in *D. basalis*, the minimum was about 86.4 % which is clearly higher than the maximum level found for *S. bruchivora*. Moreover, the maximum parasitism rate**

(number of hosts parasitized) for *S. bruchivora* was 17.6 % which is lower than the 19.1 % found in the lowest parasitism rate for both *D. basalis* populations.

The selectiveness and competitive ability of *S. bruchivora* and *D. basalis* in the presence of each other, was investigated by Mendoza (2000). He found that indeed, *D. basalis* is less selective than *S. bruchivora*. In a choice experiment on different host ages, *D. basalis* did not show preference between larvae of 16 (third larval stage), 18 (fourth larval instar) and 21 (prepupa) days old, whereas *S. bruchivora* preferred to parasitize 18 days old larvae ($p = 0.00001$). This showed that *S. bruchivora* is more specific on the appropriate host age. In addition, when infested seeds are simultaneously offered to both species (interspecific competition), more *D. basalis* individuals emerged than *S. bruchivora* (Mendoza, 2000). According to Gauthier *et al*. (1996), *D. basalis* females exhibit a wider range of oviposition behavioural plasticity in relation to the parasitoid development stage, superparasitism and the encounter rate with unparasitised hosts. Nevertheless, our experiment showed that *S. bruchivora* is not greatly affected by differences in host quality. On *Phaseolus vulgaris*, once the adult female has selected where to lay an egg, the offspring will develop successfully in that seed. This was also shown by Rios (1998) using *P. coccineus* as host plant. In contrast *D. basalis*, is not so selective and will parasitize all available host, but its performance will be affected by the seed type. So under these conditions the adult of *D. basalis* are more dependent on the host quality. Although females *D. basalis* are less selective, when they parasitize in the same seed same hosts previously parasitized by S. bruchivora, *D. basalis* showed a lower ability to develop and emerge (Mendoza, 2000). This result and its strong dependence on the quality of their host suggests that in presence of *S. bruchivora*, *D. basalis* is a poor competitor. No other study on *S. bruchivora* is available to argue in favour or against its competition ability, but *D. basalis* is well known. The lower ability of *D. basalis* females

to compete on a previous parasitized host is also demonstrated by the study of Leveque et al. (1993). These authors showed that when *D. basalis* females are introduced on a host patch 24h after the parasitoid *Eupelmus vuilleti* (Eupelmidae), multiparasitism is low, and *D. basalis* females have a low fecundity. In the reverse case, the presence of hosts previously parasitized by *D. basalis*, increase the reproduction of *E. vuilleti*, which are able to kill the *D. basalis* eggs or larvae. In this case, *D. basalis* shows an adapted evasion strategy to avoid competition, whereas *E. vuilleti* has an aggressive strategy (Van Alebeek *et al.*,1993). In competition with *Eupelmus vuilleti*, females of *D. basalis* avoid superparasitism and its density remain low (Monge *et al.*,1995; Gautier *et al.*,1999).

Conclusion:

The results of this experiment confirm i) that the domestication of the plant influences and modifies the interactions between the first and the third trophic level, and ii) that these interactions are different according to the host specificity of the parasitoids: the specialist *S. bruchivora* is more sensitive to the host quality than the generalist species *D. basalis*. Females of *S. bruchivora* seem strongly influenced by the host plant species and the choice of a good quality host to ensure the successful development of their progeny. In contrast, females of *D. basalis* parasitize the maximum number of hosts offered, taking into account the host quality to determine the sex of the progeny they produce, and the perception of the quality of the host is not altered by the original host of the female. Thus we can speculate that *S. bruchivora* and *D. basalis* have two different strategies: *S. bruchivora* being more specific, parasitizes slowly and fewer suitable good hosts (Rios, 1998). The adult female makes the choice when laying an egg and this way, ensures that the offspring will develop successfully. Whereas *D. basalis*,

the generalist, expresses a more active parasitism behaviour, to counterbalance the fact that the new generation will enable to protect itself from competitors, and strongly affected in its development by the host quality. But as mentioned by West *et al.* (1996), these experiments were done in the laboratory which cannot reflect the same effects found in the field on the relationship between the host stage (here the quality of the host plant) and the parasitoid fitness. One future prospect could be the investigation of the nature of the cues used by these two parasitoids. *S. bruchivora* seems to respond to specific cues obtained from the native host plant whereas females *D. basalis* seem to respond to more general cues, perhaps the host odour. Another important aspect could be to focus on the plant morphological characteristics and its effects on the parasitism rate. In the introduction of this chapter I talked about the loss of plant chemical compounds that may be involved in resistance against the phytophagous host, or used as cues by the parasitic wasp to locate the host. In the discussion, I asked if the higher parasitism rate on cultivated seed was not the result of a higher host density. Previous experiments showed the importance of the plant structure on parasitism rate (e.g. Gingras *et al.,* 2002). This has not been investigated in the case of the bean system. My experiment was done on seeds out of their pods. Feder (1995) showed that because the ovipositor of the parasitoid *Opius lectus* is not long enough to reach the larvae of *Rhagoletis pomonella* (Diptera), and because apples (*Malus pumila*) are larger than hawthorns (*Crataegus* spp.), flies feeding in the interior of apple fruit are more willing to escape from parasitism. Then it is possible that in case of low density of beetles in cultivated bean seeds, and even if they are a better host resource, the parasitoids are not able to locate and attack the host and as a result the number of parasitoids that emerge from fields of cultivated bean varieties could be lower than on wild beans, and the parasitoid species winning the competition could not be the same than in the laboratory

experiment. Then, as for the *Malus* system, in our system it could be interesting to consider the morphological characteristics of the plants and the presence of pods together with the morphology of the parasitoids.

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Chapter 3

Inter-population variation in host use and performance in a bruchid parasitoid

Abstract:

Many species are distributed over populations that live in a variety of habitats in which they may experience considerably different environmental conditions. For parasitoids of herbivores, the host species that they attack and the plant species that the host feeds upon, are potentially the two most important factors that will influence their fitness. Variation in these two trophic levels due to different habitat characteristics, may largely determine the type and evolution of the interaction between parasitoids and their hosts. My research examines the consequences of plant variation for the interaction between a bruchid beetle that feeds on bean seeds of the genus *Phaseolus* and one of its main parasitoids, the braconid *Stenocorse bruchivora*. Through transplant experiments I determined the performance of parasitoids that originated from beetles attacking seeds of three wild populations of *P. vulgaris*. Results showed great variation in performance among parasitoid populations. Both, the population of origin of the parasitoids and the population of origin of the seeds, influenced the performance of developing parasitoids as well as adult oviposition behaviour. It appears however, that this variation in performance among populations is decreased when parasitoids are exposed to cultivated seeds, which themselves present a more homogeneous resource for the insects.

Keywords:

Local adaptation; parasitoid; *Stenocorse bruchivora*; *Zabrotes subfasciatus*; transplant experiment; genetic variation.

Introduction:

The ability to locate a host, to parasitize it and the suitability of this host are among the most important steps in the reproduction of a parasitic wasp that ensure the maintenance of the species. To locate their host, female wasps use cues associated to the host (Godfray, 1994 for a review), and to the host plant (Turlings *et al.,* 1993; Vet *et al.,* 1995). As in all relationships between a parasite and its host, or a predator and its prey, the protagonists are closely linked and adapted to each other. This adaptation is sometimes specific to the individuals of the same localities a process known as "local adaptation". Several studies have examined the presence of local adaptation in such systems. Most of them deal only with the first and second trophic levels: plant / phytophagous host (e.g. Mopper *et al.,* 2000) or host / parasite where local adaptation is a common phenomenon (see review in Kaltz & Shykoff, 1998; Gandon *et al*., 1998). Very few studies have examined the existence of local adaptation in the interaction between the first and the third trophic level as in a plant/host/parasitoid system, but because the plant have a strong impact on the development of the parasitoid, such adaptation to the host plant by the insect of third trophic level should be exist.

For parasitoids of herbivores, the host species that they attack and the plant species that the host feeds upon, are potentially the two most important factors that will influence their fitness. Variation in these two trophic levels due to different habitat characteristics, may largely determine the type and evolution of interactions between parasitoids and their hosts. For example, populations of *Leptopilina boulardi* differ in their ability to parasitize their larval *Drosophila* host. This variation is the result of several factors such as, the number of

Drosophila species in each habitat, and the ability of these *Drosophila* larvae to escape parasitism (Campan *et al.,* in press). Habitat-related differences in parasitism behaviour have also been found for the parasitoid *Cotesia glomerata* (Nouhuys & Via, 1999). Parasitoids that originate from a cultivated cabbage field move less than parasitoids from wild populations.

Individuals of the same species can be distributed over populations that live in a variety of habitats in which they may experience considerably different environmental conditions. Than, behavioural and performance responses to the habitat stimuli may reflect genotypically fixed variation among individuals from different populations (Bouletreau & David, 1981; Kester & Barbosa, 1991; Perez-Maluf *et al*., 1998) or within populations where for example flight response to olfactory cues from infested plants vary among families of *Cotesia glomerata* and of *Microplitis croceipes* (Prevost & Lewis, 1990; Gu & Dorn, 2000).

Population differentiation in the response of parasitoid wasps to their host plant has often be studied in the context of olfactory host location. It is known that a female parasitoid may respond to the cues emitted by the plant in response to damage caused by phytophagous insects, and are able to use plant volatiles to locate their hosts. (Vinson, 1981; Turlings *et al.,* 1991; Vet & Dicke, 1992). The use of volatiles may also depend on the physiological state of the parasitic wasp (Herard *et al.,* 1988; Perez-Maluf & Kaiser, 1998) as well as on the experience of the searching parasitoid (Vet, 1983; Kaiser & De Jong, 1995). The plant can also affect the suitability of the hosts for insect parasitoids such that variation in parasitoid fitness components could be strongly influenced by the plant species or the cultivars on which the host feeds (Price *et al.,* 1980; Turlings & Benrey, 1998; Benrey *et al.,* 1998). The variability of the plant quality can also interfere with the herbivore's immune response, affecting its ability to encapsulate parasitoid eggs (Benrey & Denno, 1997; Vinson & Barbosa, 1987; English-Loeb *et al*., 1993).

The aim of this study was to determine the effect of plant variation in an interaction between a bruchid beetle (*Zabrotes subfasciatus*) and its larval parasitoid (*Stenocorse bruchivora*). By comparing three population of parasitoids that attack bruchid hosts on wild bean *Phaseolus vulgaris*, I was able to determine the relative effect of the environmental

component (plant quality) and the genetic component (population origin) on the performance and behaviour of the parasitoids. I tested the hypothesis that parasitoids from one population will perform better on hosts offered on the seeds from their own population than on seeds from a novel population.

Biological system:

Three Mexican populations were selected for this study, all of them located in the area around Mexico City. These are: Atila (18°37 N, 98°34 W) in the state of Puebla, Malinalco (18°57 N, 99°30 W) in the state of Mexico, and Tepoztlan (19°00 N, 99°07 W) in the state of Morelos. One important characteristic of these 3 populations is that they are geographically isolated from each other. They are separated by approximately 30 to 80 km**,** and located at a different altitudes which leads to a very different vegetational composition. The habitats of populations differ in size (from 3 to 6 hectares) and in the rate of infestation by bruchid beetles: the highest infestation rate is found in Tepotztlan (60%) and the lowest in Atila (19%) (Callejas, 2001). They also vary in the composition of other *Phaseolus* species and varieties. The Atila population is located in an agricultural region with cultivated beans and sugar cane fields, whereas Malinalco is situated within an archaeological site protected from human activity, and Tepoztlan is located along a road.

The host plant is the common bean *Phaseolus vulgaris* (Leguminosae: Phaseolinae). The centre of origin of this plant is located in the Mexican mountains, where the greatest diversity of wild and cultivated forms is concentrated. It has been cultivated for 7000 years in

these regions (Kaplan, 1965; Kaplan *et al.,* 1973). This makes it an interesting system, because one can find the wild and the cultivated forms growing side by side in some regions (Atila locality), while in other places we find them as isolated populations (Malinalco locality).

Zabrotes subfasciatus (Boheman) (Coleoptera: Bruchidae) is one of the main pests of field crops and stored beans in Mexico and Central America (Leroi *et al.,* 1990). It lives around 28 days at 70 % r.h. and 27 °C (Rios, 1998) and females lay an average of 40 to 50 eggs which they glue on the seed coat. First instars burrow into the bean seed where they complete their development (4 larval instars) pupate, and emerge as adults.

Stenocorse bruchivora (Crawford) (Hymenoptera: Braconidae) is a specialist, solitary ectoparasitoid of bruchids on Leguminosae plants. In Mexico, it's main hosts are *Zabrotes subfasciatus* (Boheman) (Coleoptera : Bruchidae) and *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) on beans of the genus *Phaseolus*. The females paralyse the larval host inside the bean before laying a single egg on its surface. They parasitize the third and fourth instars and occasionally pupae (Perez & Bonnet, 1984; Rios, 1998). The life cycle in laboratory is approximately 24 days at 24°C and 70 % HR. (Perez & Bonnet, 1984).

Extensive work has been conducted on the bruchids using the same populations (Callejas, 2002). This author studied the relationship between plant variation and bruchid performance on seeds originated from these 3 populations. She found that seed size varies considerably among the populations: Atila presents the larger seeds (6.16 mm +/- 0046),

whereas Malinalco presents the smallest (4.94 mm +/- 0.043). Seeds from Atila also present the largest biomass (0.60 mg +/- 0.001) compared to those from Malinalco (0.39 mg +/- 0.01). For both size and biomass, the seeds from Tepoztlan are intermediate, but significantly different from the other two. In addition, a nutrient analysis revealed a significant difference in the concentration of phosphor, and nitrogen among the seeds from the three populations. Being the highest in Atila followed by Tepoztlan and Malinalco. This study also shows that beetle performance varies among seed populations. Individuals of *Z. subfasciatus* from Atila seeds develop 20 % faster and have a higher survival (82 vs 33 %), with larger females (2.39 mm vs 2.26 mm) than bruchids from the other populations. Moreover the sex ratio is female biased in Malinalco and Tepoztlan populations (Callejas, 2002).

The fact that these three populations are geographically isolated from each other, together with the differential quality of the wild seeds, may result in local selective forces acting on the beetles and the parasitoids associated with them. Therefore I hypothesize that potential fitness differences associated with different plant populations will have resulted in adaptation by the bruchids and as a consequence, also by the parasitoids of these bruchids.

I tested the hypothesis of local adaptation in the wasp populations by investigating if parasitoids from one population are better adapted and have a higher performance on the seed from their original site (in this case on seeds from the population where they grow) than on the seeds from other populations. To test the hypothesis of local adaptation, I used a transplant experiment (e.g. Via, 1990; Sibly & Antonovics, 1991). This method has been successfully used in the study of adaptations in insects, such as the adaptation of herbivorous thrips to individual host plants, and the interaction of the leafminer (*Stilbosis quadricustella*) and it's

host plants (*Quercus geminata* and *Q. myrtifolia*) from natal sites or trees (Karban, 1989; Mopper *et al.,* 1995).

In my study, parasitoids collected from different host plant populations were reciprocally tested on the various seeds, revealing the fitness consequences of development on different hosts (Nouhuys & Via, 1999). The method allowed to estimate the relative effect of environmental and genetic components on the parasitoid performance.

To understand more about the nature of a possible genetic wasp origin effect, I conducted complementary experiment on cultivated seeds in which I performed crosses between males and females from the different wild populations. I wanted to see what was the specific role of the mother and the father in the different variables measured. To do that, I examined the larval performance of hybrid offspring using hosts reared on cultivated seeds. The use of hybrids to evaluate the genetic component on the behaviour and performance in the study of local adaptation can be very effective. Fleury *et al.,* (1995) used reciprocal genetic crosses to study local adaptation in the foraging behaviour of two populations of the parasitoid *Leptopilina heterotoma*, and demonstrated a genetic basis of the variation in locomotor activity rhythm. They also correlated this behaviour with various selective pressures (day length, quality and nature of hosts available such as, species diversity and abundance).

Material and methods:

Collection of seeds and rearing of bruchids and parasitoid:

Individuals of *Stenocorse bruchivora* (Hymenoptera : Braconidae) were obtained from wild seeds of *Phaseolus vulgaris* infested with parasitized bruchids. Once collected in the field, seeds were placed in an aquarium (25x15x15 cm) until emergence of the wasps. The newly emerged adults (G0) were placed in second aquarium with food (sugar, water and honey) and a mixture of wild bean seeds infested by 21 days old *Zabrotes* larvae (to obtain the wasps for the transplant experiment, G1) or black beans (cultivated variety) infested by 18 days old *Zabrotes* larvae (to obtain wasps for the hybrid experiment). The mixture of wild

seeds from the different populations was used to reduce any host related maternal effect. Differences in the age of *Zabrotes* larvae used between wild and cultivated beans is to account for differences in development time in these seeds. In wild seeds, larval development is slower compared to cultivated beans (Rios, 1998; personal observation).

Wild bean pods were collected in the three localities and all seeds containing eggs and entrance or emergence holes of other bruchids (*Zabrotes subfasciatus* and also *Acanthocelides* spp.) where discarded from the experiment.

To avoid a possible influence of the quality of the host, I used individuals of *Zabrotes subfasciatus* from a laboratory colony. This way, any differences found between treatments would indicate an effect from the origin of the wasps and / or the origin of the seeds, but not the origin of the bruchid. All these experiments were done in an environmental chamber at 27°C with a photoperiod of 16L:8D.

Experimental designs:

Local adaptation experiment:

Every day a newly emerged couple from the first generation (G1) was placed in a gelatine capsule during half a day to assure mating. After this step, all wasps from the same population were placed in a plastic box with food to ensure mating (if one male was sterile or unable to mate the female in the gelatine capsule). After 4 days, each female was placed in a plastic box (∅ 4.5 cm) together with a random male and with 10 or 20 wild seeds infested by one 21-days-old *Zabrotes* larva. To stimulate parasitism, each plastic box was covered with a piece of nylon, and placed in an aquarium full of female wasps. I studied the behaviour of the

mother and measured the following fitness components on the progeny: number of newly emerge parasitoids (G2), sex ratio, size and development time.

As a component of wasp behaviour I analysed the "female motivation" which is the number of females that parasitized hosts in the seeds offered, which differs from the parasitism rate, calculated as the number of seeds parasitized by each female. It is however not a direct behavioural observation.

This experiment was conducted in two different ways. In the first one, a single female of *S. bruchivora* was placed in a plastic box with 10 wild seeds and one male, during 3 days. In the second experiment the same procedure was followed, but this time with 20 wild seeds in a box and a 6-days exposure period. The experiment with 10 seeds / 3 days (exp. A) was done to obtain a more accurate estimate of the development time of the new generation; the experiment with 20 seeds / 6 days (exp. B) was done to maximize the number of emerging parasitoids in order to measure their size. I tested between 13 to 22 couples per combination in experiment A, and between 4 and 8 couples in experiment B (see Table 1). This relative low number of tested couples was due to a mite infestation that killed 90% of the G1 insects right before or within two days after their emergence.

Because some of the insects failed to emerge, seeds were dissected and the parasitism rate and the sex ratio, were registered using the total of individuals that emerged from the seeds and those obtained from the dissection. Parasitism rate was estimated as the number of parasitized hosts (from which new wasps emerged) divided by the total number of hosts offered. For this measurement, I considered only the replicates from which wasps emerged. Replicates that did not produce any wasps were discarded from the analysis. Sex ratio was estimated in two ways: in the first I used individuals obtained from all the replicates

while in the second, I only used individuals that emerged from boxes that produced parasitoids of both sexes. This second approach was used in order to avoid biasing the sex ratio analysis by using virgin females. Tibia length was used as an index of parasitoid size. For this, I pooled all the individuals from both experiments (A and B). For both experiments A and B, the development time of the parasitoids was determined from the third day of the exposure period.

Experiment	Wasp origin	Seed origin	Name	Number of tested couples
A	Atila	Atila	Aa	17
A	Atila	Malinalco	Am	17
A	Atila	Tepoztlan	At	18
A	Malinalco	Atila	Ma	18
A	Malinalco	Malinalco	Mm	17
A	Malinalco	Tepoztlan	Mt	13
A	Tepoztlan	Atila	Ta	22
A	Tepoztlan	Malinalco	Tm	13
A	Tepoztlan	Tepoztlan	Tt	17
B	Atila	Atila	Aa	7
B	Atila	Malinalco	Am	8
B	Atila	Tepoztlan	At	7
B	Malinalco	Atila	Ma	6
B	Malinalco	Malinalco	Mm	6
B	Tepoztlan	Atila	Ta	6
B	Tepoztlan	Malinalco	Tm	4
B	Tepoztlan	Tepoztlan	Tt	7

Table 5: number and treatment of the tested couples and in each experiment. Experiment A: one female / 10

seeds / 3 days; Experiment B: one female / 20 seeds / 6 days.

Hybrid experiment:

For this experiment common black beans (cultivated variety) were offered to the parasitoid females (G0). Each bean was infested with one to three 18 days-old *Z. subfasciatus* larvae. Three days prior to the emergence of the wasps, all black beans were individually placed in a plastic capsule. In this study I controlled any possible effect of the bruchid host (all *Z. subfasciatus* individuals came from the laboratory colony), and of the host plant because all the bean seeds were the same (the same variety of cultivated beans).

Every day newly emerged wasps were collected. Females (G1) were individually isolated in a gelatine capsule with one male from the same or a different population to obtain full sib offspring (if female and male wasps came from the same population), or half sib offspring (if both the mother and the father came from different populations). After 24 hours all males and females from the same batch were placed in a plastic box for 4 days with honey as a food source. In this way, I assumed that each female was mated by a predicted male. After this period one couple was placed in a plastic box (base ∅3.5 cm, top ∅ 4.5 cm) with 3 black beans, infested each by three 18 days old *Z. subfasciatus* larvae. I allowed females to parasitize for a period of 3 days. As for experiment A, development time of the new wasp generation was determined from the third laying day. To obtain the parasitism rate and the sex ratio, I dissected all the seeds at the end of the experiment. The tibia length of each adult parasitoid was also recorded, using an ocular micrometer.

Statistical analysis:

Because of the low parasitoid emergence rate, I did not get offspring from all treatments (wasp origin: A,T,M / seed origin: a, t, m combination). In the experiment A (10 seeds), Ta, Tt, and Ma groups did not produce G2 wasps. In the experiment B (20 seeds), the Mt and Mm treatment were missing due to logistic problem or lack of emergence (see Table 1 for the exact reference to the treatments). Thus, in the statistical analyses these treatments

were dropped.

Because the data did not meet the assumptions of homogeneity of variance, non-parametric methods were used. To investigate the differences between treatments I did a Kruskal-Wallis tests completed with a Wilcoxon Rank test to examine the effect of seed type and wasp strain, on the parasitism rate, sex ratio, development time and tibia length. The "motivation" behaviour was analysed by a contingency table. (Sokal & Rohlf, 1995)

Results:

For each parameter and for each treatment, the sample size tested is showed in Table 2.

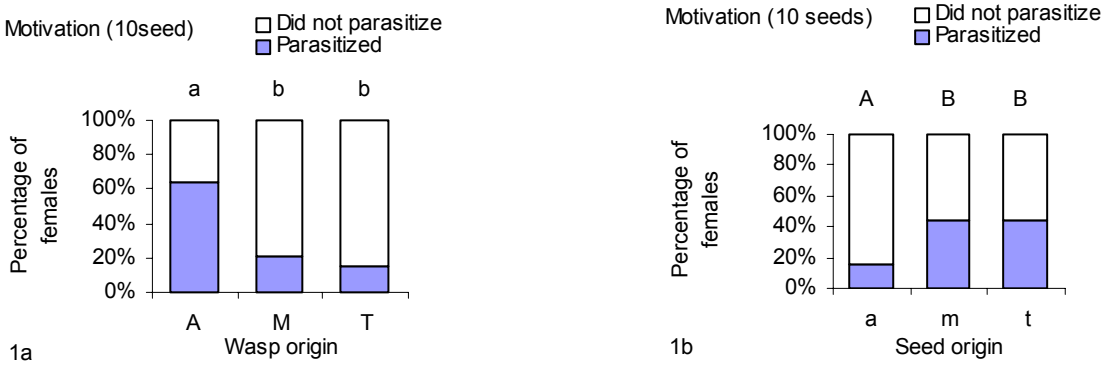
	A: 10 seeds / 3 days of exposure								
	Aa	**At**	**Am**	**Ta**	**Tt**	**Tm**	**Ma**	**Mt**	**Mm**
Motivation	17	18	17	22	17	13	18	13	17
Parasitism rate	8	14	11	0	0	7	2	6	3
Sex-ratio	5	12	7	0	0	5	0	6	3
Development males	13	13	14	0	0	9	0	11	3
Development females	8	27	21	0	0	8	0	13	4
	B: 20 seeds / 6 days of exposure								
	Aa	**At**	**Am**	**Ta**	**Tt**	**Tm**	**Ma**	**Mt**	**Mm**
Motivation	7	7	8	6	7	4	6	0	6
Parasitism rate	7	6	7	3	4	2	4	0	0
Sex-ratio	5	6	5	2	3	2	2	0	0
Development males	21	14	37	5	10	9	5	0	0
Development females	39	46	32	2	14	6	15	0	0
	A & B								
	Aa	At	Am	Ta	Tt	Tm	Ma	Mt	Mm
Tibia length males	35	28	53	6	8	18	5	11	3
Tibia length females	46	73	59	3	18	14	16	13	4
	Hybrid study								
	M*m	T*t	A*a	M*t	M*a	T*m	T*a	A*t	A*m
Motivation	15	13	14	13	14	13	13	15	12
Parasitism rate	10	12	14	13	14	12	13	13	10
Sex-ratio	10	12	14	13	14	12	13	13	10
Development males	5	11	25	11	23	12	28	21	10
Development females	10	10	13	20	5	7	12	7	11
Tibia length males	10	17	34	24	21	18	33	27	24
Tibia length females	24	26	26	30	6	9	19	7	12

Table 6: Sample size (n : number of individuals) used for the analysis among parameters for each treatment.

Adult parasitoid behaviour: the females "motivation" is the percentage of females that parasitized in the different treatments.

Experiment A: 1 female / 10 wild seeds / 3 days of exposure:

A first pattern that emerges is a wasp origin effect: Atila females were more motivated to parasitize than females from the other two populations ($p < 0.0001$) (Fig 1a). There was no significant difference between females from Tepoztlan and females from Malinalco ($p = 0.478$). There was also a significant effect of the origin of the seeds on the percentage of females that parasitized the hosts ($p = 0.0016$) (Fig. 1b). Overall, whenever the seeds from Atila were present, significantly fewer females parasitized the hosts than when the seeds from the other populations were offered. The percentage of females that parasitized was not significantly different between Tepoztlan and Malinalco ($p = 0.9272$).

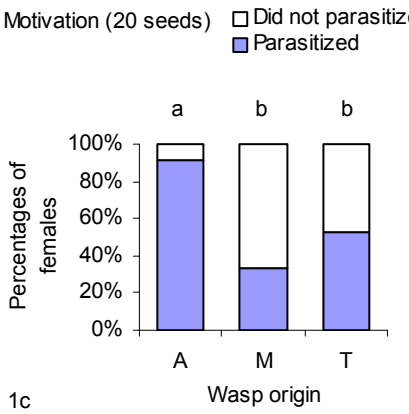


Figures 9a & 1b: Motivation: proportion of females that parasitize the seeds in experiment A: one female / 10 wild seeds / 3 days of exposure; (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; seed origin: a: Atila, t: Tepoztlan, m; Malinalco). Different letters indicate significant differences between the wasp origin or seed

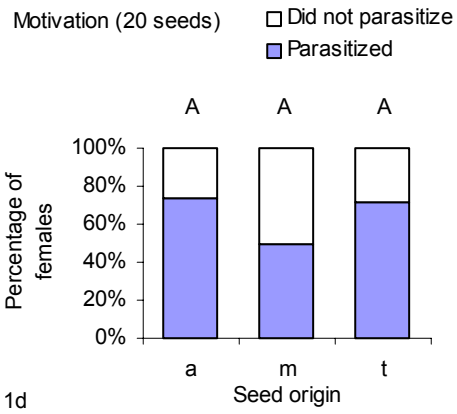
origin.

Experiment B: 1 female / 20 wild seeds / 6 days of exposure:

As in the previous experiment, I also found a significant effect of the wasp origin on the proportion of females that parasitized the hosts ($p = 0.0016$). Here again the females from the Atila population were more willing to parasitize than the females from the two other populations (Fig. 1c). There was no significant difference between females from Tepoztlan and females from Malinalco ($p = 0.2957$). In contrast with the results from the previous experiment, when 20 seeds were offered for a period of 6 days, there was no significant effect of the seed origin on the motivation to parasitize ($p = 0.2655$) (Fig 1d). The difference in the results of these two experiments confirms that by exposing parasitoids to the different seeds for a longer period of time, the possible effects of seed type are erased and more females agree to parasitize the hosts.



1c



1d

Figure 1c & 1d: Motivation: proportion of females that parasitize the seeds in experiment B: one female / 20 wild seeds / 6 days of exposure; (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; seed origin: a: Atila, t: Tepoztlan, m; Malinalco). Different letters indicate significant differences between the wasp origin or seed origin.

Parasitoid performance:

Parasitism rate

The hypothesis of local adaptation predicts that wasp would parasitize a higher proportion of hosts on seeds from their own locality that on seeds from other localities. There are significant differences between the different seed origin * wasp origin combinations (treatments) in the number of hosts parasitized by the females, but this differences do not reveal evidence of local adaptation (Fig.2a).

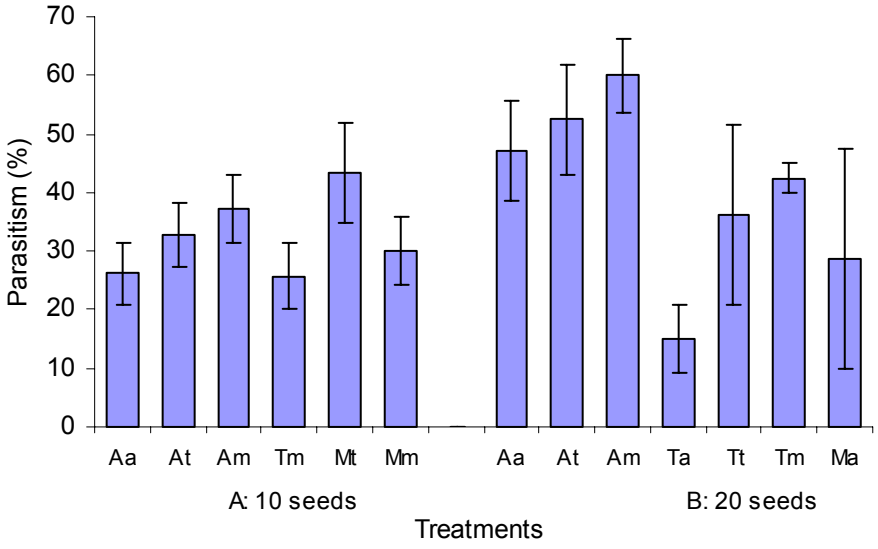
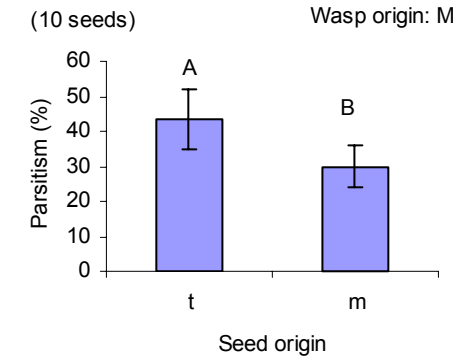


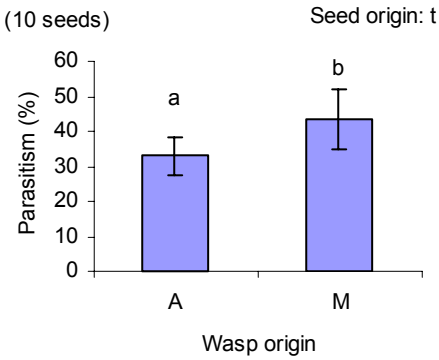
Figure 10a: Effect of the wasp origin and seed origin on parasitism in experiments A & B**.** (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

Experiment A: 1 female / 10 wild seeds / 3 days of exposure:

Wasps that originated from Atila did not parasitize more hosts on seeds from Atila than host on seeds from Tepoztlan and Malinalco (p = 0.58). Moreover females originated from Malinalco parasitized more host on the seeds from Tepotztlan than on seeds from their own locality (p = 0.0003) (Fig. 2b). On seeds from Malinalco, no difference was observed between the wasp origin (A, T, M) (p = 0.37), but when seeds from Tepoztlan were offered, females from Atila appeared to parasitize more than females from Malinalco (0.0001 > p) (Fig. 2c).



2b



2c

Figures 2b & 2c: Effect of the wasp origin or seed origin on parasitism in experiment A. Different letters indicate significant differences between the wasp origin or seed origin. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

Experiment B: 1 female / 20 wild seeds / 6 days of exposure:

Here again there was no evidence for local adaptation: females from Atila and Tepoztlan did not parasitize more hosts on seeds from their original locality (Atila: p = 0.54; Tepoztlan: p = 0.39). When the hosts were offered in seeds from Atila, there was no significant difference in parasitism rate between the different wasp origins (A, T,M) (p =

0.18). In seeds from Tepoztlan (Fig. 2d) and Malinalco (Fig. 2e), the females that originated from Atila parasitized more than females from Tepoztlan ($p < 0.0003$).

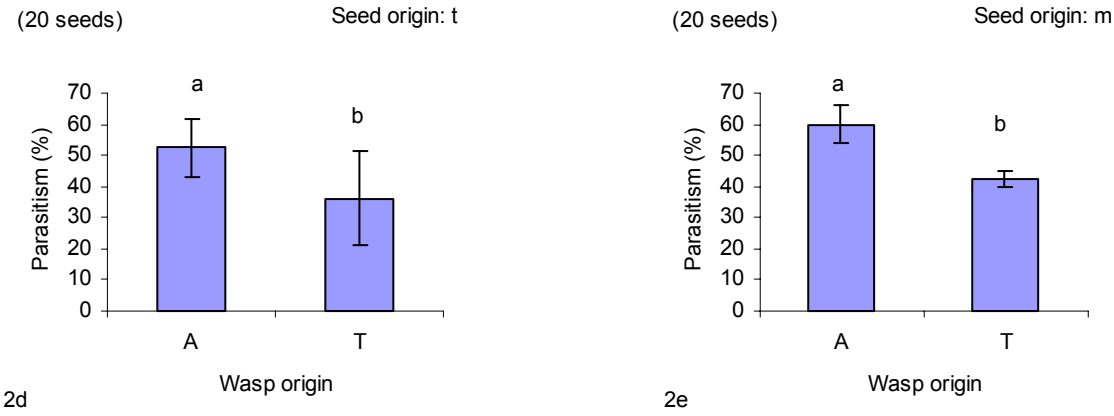


Figure 2d & 2e: Effect of the wasp origin or seed origin on parasitism in experiment B. Different letters indicate significant differences between the wasp origin or seed origin. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

Sex-ratio:

In none of the experiments (10 seeds: Fig. 3a; 20 seeds: Fig. 3b), I found a significant effect of the seed origin and wasp origin on the sex ratio (see Table 3 for review of *p* values). Again, if there was local adaptation, one would expect that wasps from Atila for example, will produce more female offspring on seeds from Atila, and that was not the case.

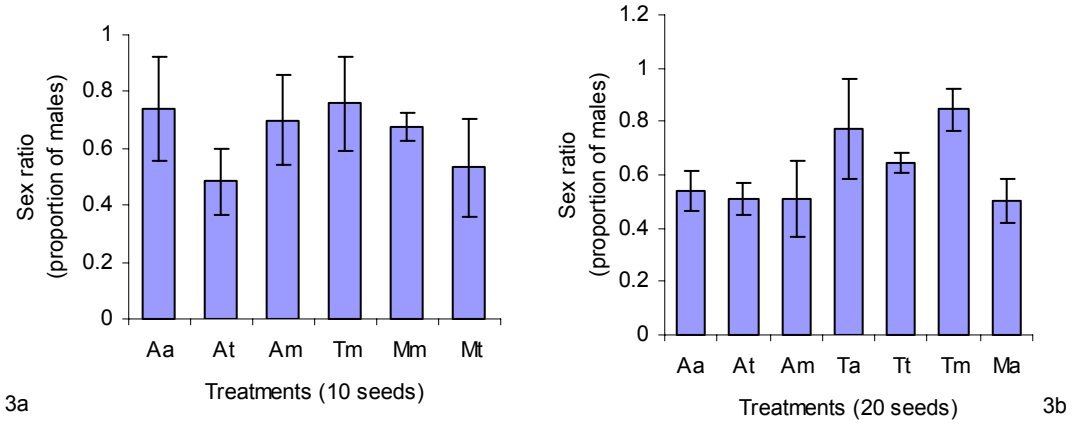


Figure 11: Sex ratio: proportion of males in experiment: A: 3a) one female / 10 wild seeds / 3 days of exposure; 3b) and B; one female / 20 wild seeds / 6 days of exposure. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

P values		Sex ratio total	Sex ratio corrected
A: 10 seeds	wasp origin	0.6535	0.0766
	seed origin	0.3448	0.9809
B: 20 seeds	wasp origin	0.2994	0.24
	seed origin	0.2689	0.6458

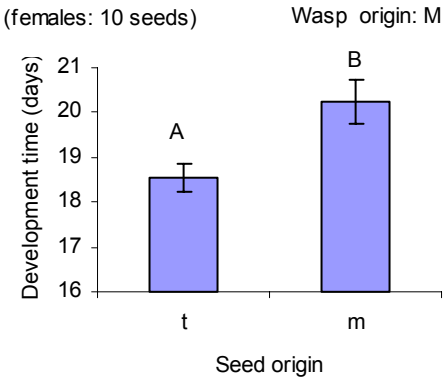
Table 7: P values for the different tests used to analyse the wasp and seed origin effects on the sex ratio in experiment A: one female / 10 wild seeds / 3 days of exposure; and B: one female / 20 wild seeds / 6 days of exposure.

Larval development time:

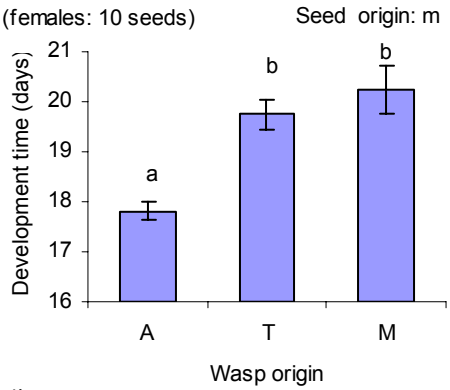
Experiment A: 1 female / 10 wild seeds / 3 days exposure:

Again, no evidence for local adaptation for larval development time was found. The

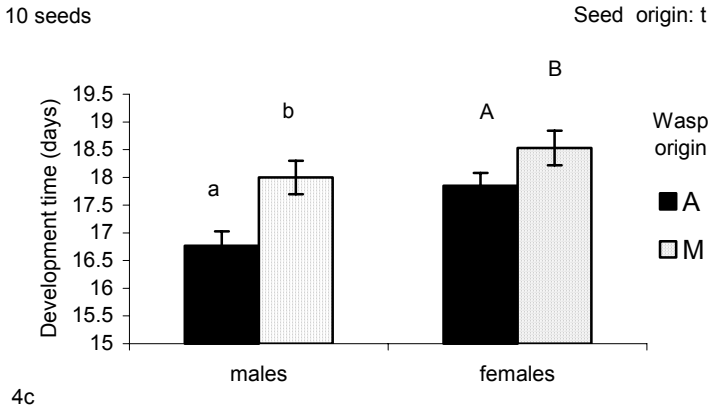
offspring from Atila did not develop faster on seeds from which their mother came from (male: p = 0.18; females: p = 0.51). Moreover, females from Malinalco needed more time on the seeds from Malinalco to achieve their development than on seeds from Tepoztlan (p < 0.0001) (Fig. 4a). However I found a significant effect of the wasp origin on the larval development time of both males (p = 0.0152) and females (p < 0.0001). Development was faster for males and females from Atila than other strain origins, both in seeds from Tepoztlan (Fig. 4b) and in seeds from Malinalco (Fig. 4c).



4a



4b



4c

Figures 12a, 4b & 4c: Larval development time of the males and females on experiment A : one female / 10 wild seeds / 3 days of exposure. Different letters indicate significant differences between the wasp origin or seed origin. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

Experiment B: 1 female / 20 wild seeds / 6 days of exposure:

Conclusion

Here again there was no evidence for local adaptation. Males from Atila develop slower in seeds from Atila than in seeds from Tepoztlan ($p = 0.045$) (Fig. 5a). No significant difference was found on the development time of females from Atila on seeds from the three populations ($p = 0.20$). And, no significant difference was found for males from Tepoztlan tested on the three seed populations ($p = 0.14$). Males from Atila develop faster than males from Tepoztlan ($p < 0.0001$) in seeds from Tepoztlan (Fig 5b) and in seeds from Malinalco (Fig.5c). But no differences among wasp strains were found on the development time of males in seeds from Atila ($p = 0.17$). For females however, there was a significant difference due to the wasp origin when females were tested in seeds from Atila (Fig. 5d): females from Malinalco develop slower than females from the other 2 populations ($p < 0.0001$). Females from Atila develop faster than females from Tepoztlan on seeds from Tepoztlan (Fig. 5b), and seeds from Malinalco (Fig. 5c), which confirms the lack of local adaptation also for females from Tepoztlan ($p < 0.0001$).

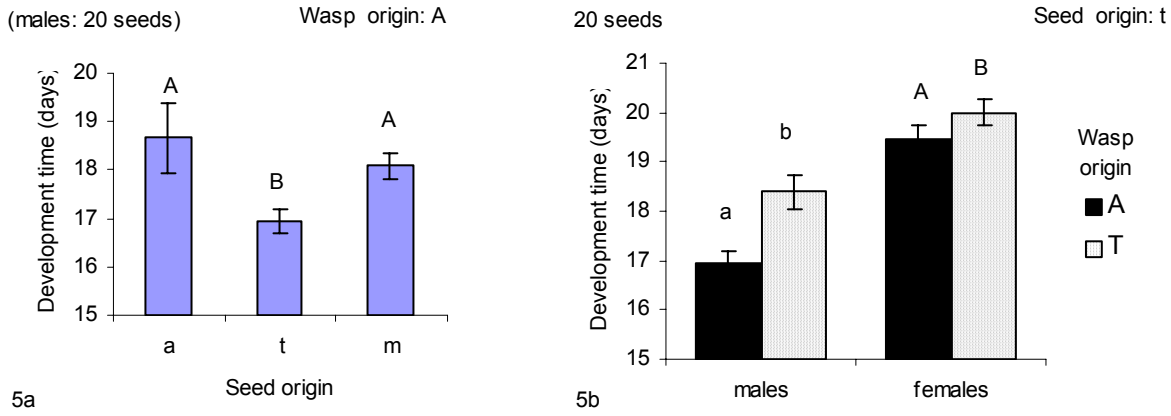


Figure 13a & 5b: Larval development time of the males and females on experiment B : one female / 20 wild seeds / 6 days of exposure. Different letters indicate significant differences between the wasp origin or seed origin. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

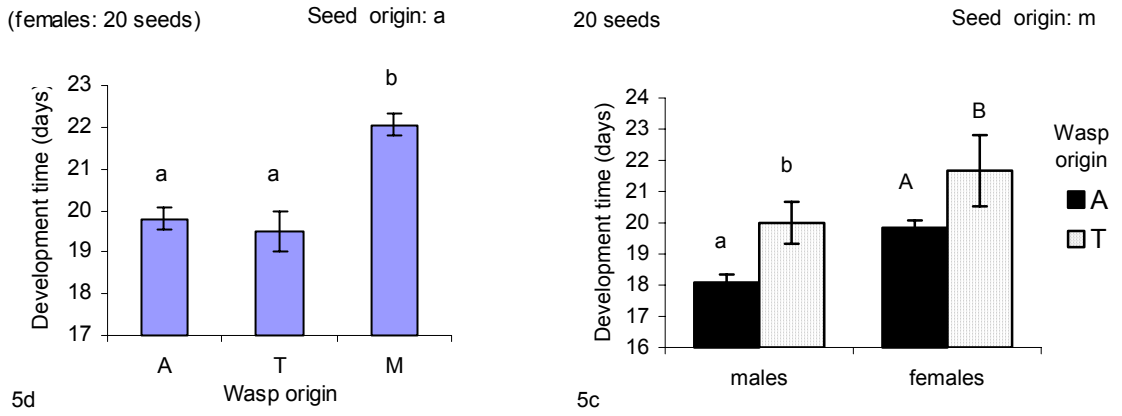
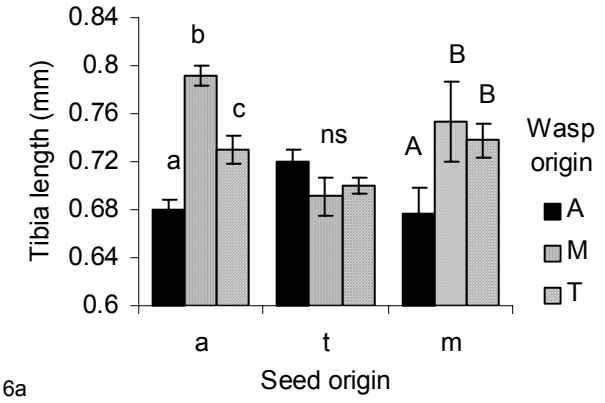


Figure 5c & 5d: Larval development time of the males and females on experiment B : one female / 20 wild seeds / 6 days of exposure. Different letters indicate significant differences between the wasp origin or seed origin. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; Seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

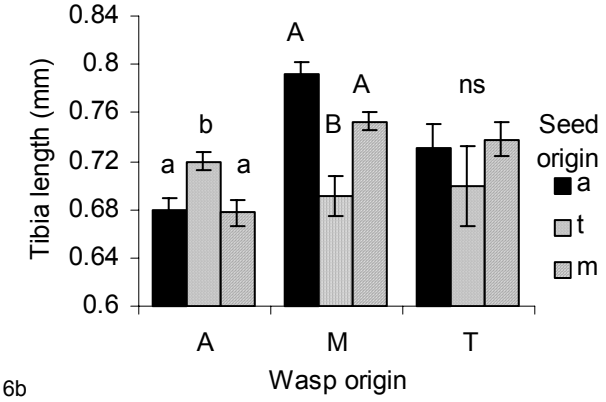
Tibia length:

Data from both experiments were pooled for this analysis because there were no significant differences between them ($0.084 < p < 0.66$). In the case of the males, there was an effect of the wasp origin and of the seed origin on the size of the emerging parasitoids, but no evidence of local adaptation(Fig. 6a). Atila males are smaller than other males on Atila seeds ($p = 0.004$). No difference was found between the different wasp populations in seeds from Tepoztlan ($p = 0.21$). On seeds from Malinalco, the males from this same population are larger than males from Atila ($p = 0.033$), but there are not significantly different from the males of Tepoztlan ($p = 0.33$) (Fig. 6b). Significant difference between the wasp origin, mainly between the males of Atila, smaller than the males of Malinalco, was found on Atila and Malinalco seeds, but not on the Tepoztlan seeds ($p = 0.21$). Except for the males originated from Tepoztlan, significant differences were found according to the seed origin (Fig. 6b). Males Atila are larger on Tepoztlan seeds ($p = 0.0094$), whereas males from

Malinalco are smaller on these seeds than on the two other seed origins ($p = 0.0048$). In the case of the females (Fig. 7), no significant differences were found according to the wasp origin or the seed origin ($0.29 < p < 0.98$).

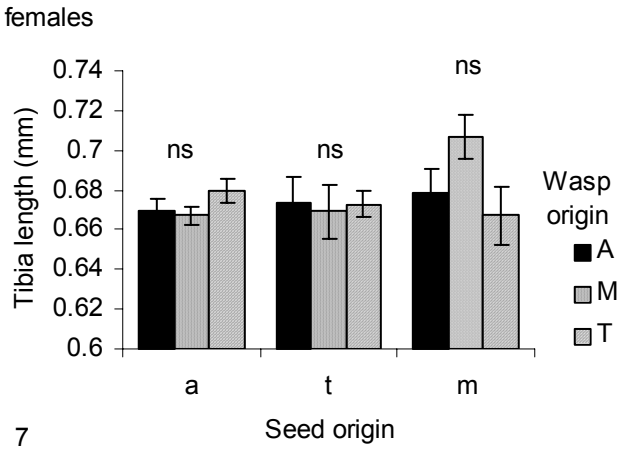


6a



6b

Figures 6a & 6b: 6a: Male tibia length according the seed origin. 6b: Male tibia length according the wasp origin. Different letters indicate significant differences between the seed origin or wasp origin. (Seed origin: a: Atila, t: Tepoztlan, m; Malinalco; Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco)

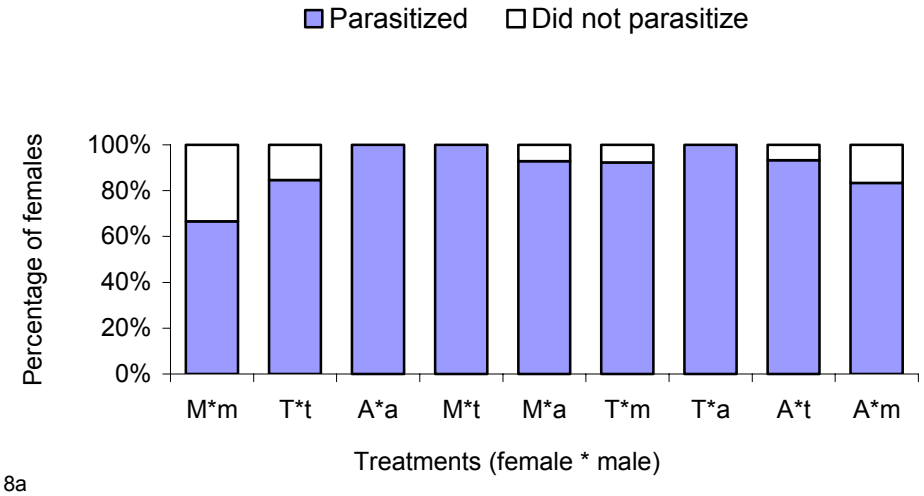


7

Figure 7: Female tibia length according the seed origin. Different letters indicate significant differences. (Wasp origin: A: Atila, T: Tepoztlan, M: Malinalco; seed origin: a: Atila, t: Tepoztlan, m; Malinalco).

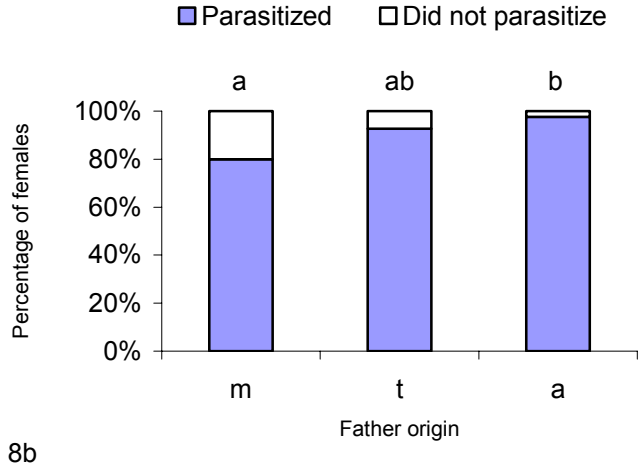
Hybrid experiment:

For the "motivation" behaviour, the results obtained from this experiment are very different from those in the previous one done with wild seeds, even if the females that originated from the population of Atila were more willing to parasitize the seeds than females that originated from the two other populations (Fig. 8a). No differences were found among the females from the three populations ($p = 0.49$). It is important to note that the seeds used in this experiment were cultivated which provide a higher quality resource than wild seeds. The lack of differences between treatments allowed me to pool the data with respect to the female origin or the male origin. In this case, one unexpected result was that males had an influence on the female behaviour to which they are mated ($p = 0.024$). Females mated with a male originated from Malinalco are less willing to parasitize than females mated with males originated from Atila ($p = 0.0119$) (Fig. 8b). There was no influence of the female origin when treatments were pooled ($p = 0.49$).



8a

Figure 8a: Motivation: proportion of females that parasitized the cultivated seeds (A: Atila, T: Tepoztlan, M: Malinalco; female * male).



8b

Figure 8b: Percentage of female that parasitized the seed according to the father origin (m: Malinalco; t: Tepoztlan; a: Atila). Different letters indicate significant differences between the father origin.

For parasitoid performance, I only found a significant effect of the parent origin on the development time of the offspring. For the male offspring, the origin of the mother had a significant effect on their development ($p < 0.0001$) (Fig. 9), but not the father origin, which

is expectable because males are haploid and do not participate into the male production. Sons from Atila mothers developed faster than males from the other two populations, and sons from Malinalco mothers developed slower than the other males. Finally, sons from Tepoztlan mothers had an intermediate larval development time but significantly different from the others ($0.0001 < p < 0.017$) .

For the female offspring, both the population of origin of the father and the population of origin of the mother had a significant effect on the larval development time (father origin : $p = 0.0330$, mother origin: $p < 0.0001$) (Figures 10 & 11). Offspring that came from mothers or fathers from Atila developed the fastest, and daughters that came from fathers or mothers from Malinalco developed slower. In the case of offspring from Tepoztlan, the father influence is different than the mother influence. When the daughter come from Tepoztlan father, they develop faster than daughter from Atila ($p = 0.017$) but did not differ from daughter from Malinalco ($p = 0.23$) (Fig. 10). Daughters from a Tepoztlan mother need significantly less time to develop than daughters from Malinalco ($p = 0.0023$), but did not differ from daughters from Atila ($p = 0.26$) (Fig.11).

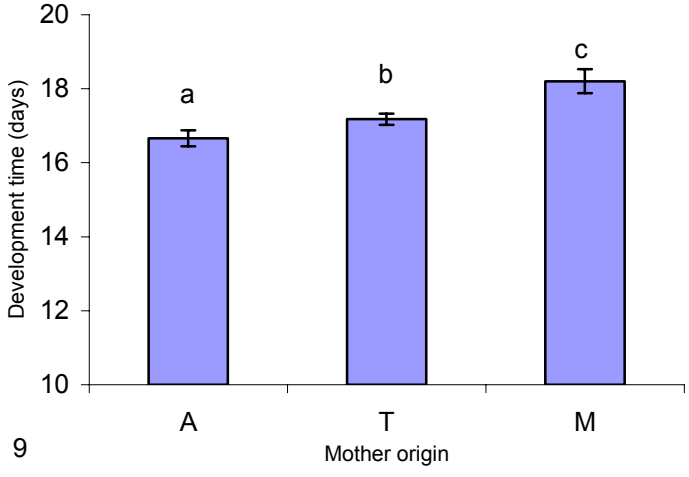
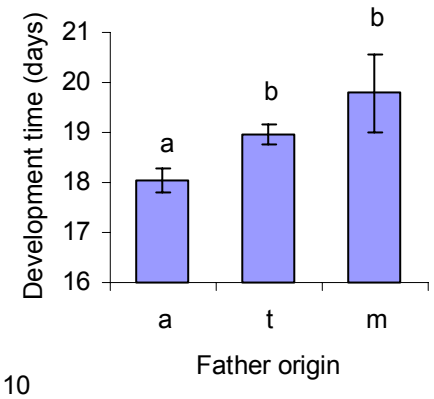


Figure 9: Mother origin effect on the development of the "hybrid" males on cultivated seeds (A: Atila, T: Tepoztlan, M: Malinalco). Different letters indicate significant differences between the wasp origin.



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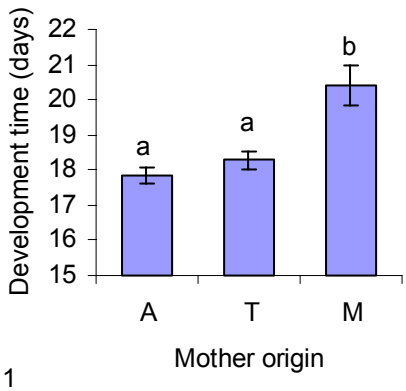

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Figures 10 & 11: Father and mother origin effects on the larval development time of the hybrid females (Father origin: a: Atila, t: Tepoztlan, m; Malinalco; Mother origin: A: Atila, T: Tepoztlan, M: Malinalco) . Different letters indicate significant difference.

The parasitism rate, the sex-ratio and the parasitoid size did not reveal significant differences due to the parent's origin. The population of origin of the mother and of the father did not have an influence on these variables when the hosts were offered on cultivated seeds (Table 4 for *p* values).

	Hybrids p values	
	Mother origin	Father origin
Parasitism rate	0.1274	0.7161
SexRatio total	0.2606	0.1137
SexRatioCorrect	0.142	0.6454
Male Size	0.5137	0.6169
Female Size	0.2518	0.3202

Table 8: P values used for the different tests to analyse the different fitness components of the hybrids.

Discussion:

The transplant experiment was conducted to investigate the potential for local adaptation of parasitoid populations (third trophic level) to the host plant (first trophic level) of their population of origin. The results did not provide evidence for the existence of local adaptation, but they showed behavioural and performance differences among the parasitoid population with respect to their origin and the origin of the seeds in which they developed.

Although there was no evidence for local adaptation in the motivation of females to parasitize when seeds from their own population were offered, one interesting finding was that, in the presence of infested seeds, females that originated from Atila were more willing to parasitize compared to the females originated from the other populations. This result is independent of the number of seeds and the time allowed for parasitism, and it is confirmed by the greater parasitism rate exhibited by these females during the 20 seeds experiment.

Not only females from the Atila populations showed a higher motivation to parasitize the seeds, but also the development time of their offspring is shorter. However their male offspring is smaller, perhaps as a consequence of shorter development time. In the analysis of performance of the bruchid beetles *Zabrotes subfasciatus* originated from the same populations than those used in the present study, the Atila population also differed from the others. This population was the only one showed local adaptation. For almost the parameters of individuals *Z. subfasciatus* were better: greater number of eggs, greater survival, smaller development time (Callejas, 2002).

The origin of the seeds also affected the behaviour and performance of the parasitoids. But this effect differed between wild and cultivated seeds. On wild seeds, the differences between the wasp strains were clear, and within each wasp strain, the seed origin induced differences: females were less willing to parasitize on the Atila seeds, larval development time was shorter on Tepoztlan which also influence the male size. On cultivated seeds where only one seed origin was used, the differences between the wasp origins were found only on the motivation of the females to parasitize and on development time of offspring.

In the 10 seed experiment, it appears that the seeds from Atila are less attractive to the parasitoids, but not in the 20 seeds experiment. Although I still cannot explain this result, I believe that it may be due to the quality of the seeds. The seeds from Atila were of better quality for the bruchids than the seeds from the other populations (Callejas, 2002). Because the bruchids develop faster in these seeds, they could have been already at the stage of pupae when the seeds were offered in the 10 seed experiment, and therefore, were not attractive for the parasitoid, which prefers to parasitize 4th instar larvae. In contrast, in the 20 seeds experiment, the lack of this effect can be explained by the longer period of time during which the females were allowed to oviposit. A female can delay her oviposition if the host quality is no adequate but after some days, if the female wasp does not encounter suitable host, she has to parasitize whichever hosts are available. Although females of *S. bruchivora* prefer to parasitize the 4th instar of *Zabrotes subfasciatus*, they can also parasitize pupae (Perez & Bonnet, 1984; Rios, 1997). So that, during the 6 days of the experiment, the female could not escape from the experimental box and was forced to parasitize the seeds from Atila, which probably contained pupae and not 4th instar.

The offspring from the Atila population tend to develop faster than the offspring from

the other wasp populations. However, their development time is longer on the bruchids within the seeds from their own population. If the seeds from Atila are not suitable for the development of the wasp (which is the reverse case for the bruchid host), the wasps maybe adapted to develop with a great constraint (they increase their larval development time to compensate for the poor host quality of the bruchids from Atila seeds), which results in a faster development time, and a larger size (for the males) on seeds from other populations (Malinalco and Tepoztlan seeds). This potentially lower seed quality in the Atila population, may also explain why Atila females are more willing to parasitize, and parasitize more hosts to compensate the bad performances of their offspring on the seeds from Atila. However there is no indication that the poor host quality of the Atila seed had an effect on the fitness of the other wasp population, a fact that talks against the argument above.

In general, parasitism rate in these experiments was very low (maximum 43 % in the 10 seeds experiment). This low parasitism rate can be due to a lack of oviposition experience of the females. Oviposition experience can reduce the variability in oviposition behaviour and increase the probability of active foraging (Vet *et al.,* 1990). In the present experiment this was not done because giving an oviposition experience would be an artefact that could have confounded the genetic differences of behaviour of these wasps. However, this experiment confirm the result found in the previous chapter where this parasitoid species expressed a lower parasitism rate in comparison to the generalist *Dinarmus basalis* (Hymenoptera: Pteromalidae).

The results from this study confirm the importance of investigating the genetic and environmental components on the interactions between parasitoids and their hosts. Both the origin of the parasitoids and the origin of the seeds, influenced the performance of developing parasitoids as well as their oviposition behaviour.

It appears that independent of variation in the seeds from the different populations, other factors, such as, variation in the ability of the parasitoids to successfully attack the

bruchids are also responsible for the differences found in performance among parasitoid populations. A genetic component in the foraging behaviour of a parasitic wasp was demonstrated also in *Cotesia congregata* (Kester and Barbosa, 1994), where two populations (and hybrids obtained from reciprocal crosses) did not forage equally on tobacco and tomato plants, as a result of their population of origin. The "tomato population" (Wye) preferred to parasitize hornworms on tomato rather than on tobacco and had a longer searching response on tomato. Whereas the "tobacco population" (Upper Marlboro) showed no preference of host for parasitism, but expressed a prolonged searching behaviour on tobacco plants.

In a transplant experiment with *Cotesia glomerata*, Van Nouhuys & Via (1999) found that the behaviour of the wasp was also influenced by the wasp origin (wild and cultivated habitat), but they concluded that it was not related to local adaptation. In this context, the parasitism rate was higher in the wild habitat than in the cultivated one.

In our system, all the wasps came from wild field populations. The main difference between these populations is the amount of resource available: Malinalco offers a larger density of *Phaseolus* plants in a small area, while the host plants are more dispersed and scarce in Atila. A possible explanation of the higher activity of wasps that originated from Atila may be due to this different plant layout. A female has to fly more and loose more time to find an adequate host plant that will carry a suitable host. Added to this problem, due to the better quality of the seeds, the *Zabrotes* beetles develop faster on these seeds (Callejas 2002). That means that the window of vulnerability for larvae of the right instar for the parasitoids is narrower in comparison to Malinalco. I believe that in order to compensate for these two factors, females from Atila have to be more active. This may explain why they were more willing to parasitize the wild seeds and this behaviour may be "locally adapted". A similar

adaptative behaviour has been found in *Leptopilina boulardi*, a parasitoid of frugivorous *Drosophila* (Campan *et al.*, in press). In response to fruit odours, two different populations behave differently in their probing response. The activity pattern to probe the substrate to parasitize host is genetically transmitted. There is both nuclear and cytoplasmic influence. The hyperactivity of the "Brazzaville" strain was coherent with specific reproduction constraints: a low ability to suppress the host immune defence compared to the Nasrallah strain and could be viewed as the result of a locally adapted behaviour.

Also in this case of *S. bruchivora*, I found evidence for a genetic component probably transmitted by both parents. Hybrid wasp behaviour should be investigated on wild seeds and it will be useful to demonstrate if there is a genetic component to the wasp oviposition behaviour. Differences in behaviour can be found not only at the population level but also within a population. In *Trichogramma brassicae* (Wajnberg, 1994) different behavioural strategies are expressed in the same population by wasps that colonize the same host patches. This indicates that a genetic component may not be the only factor responsible for a differential behaviour, but other factors such as, previous experience and associative learning may explain different behaviour among individuals from a same population (Vet, 1983; Turlings *et al.,* 1993; Kaiser *et al.,* 1995).

An interesting point that arises from this study is some indirect evidence of a loss of genetic variation as a result of plant domestication. The use of cultivated seeds decreased the differences in the behaviour and performance of the parasitoids from the different populations. This result illustrates the importance of conducting studies using a setting as close as possible to the natural environment, in this case, wild seeds.

In conclusion, this study is an illustration of the complexity of the behaviour and performance of insects in natural systems in which many agents act upon each component of fitness, and where the constraints may differ between populations. The results show that the ability of the wasps to parasitize and the performance of developing parasitoids, are influenced by the origin of their parents, and also by the origin of the seeds in which their hosts are reared. Thus there is a genetic and an environmental component that results in differential behaviour and performance of parasitoids from the different populations. But I did not find evidence of local adaptation in performance for the parasitoids as it was showed for the bruchid host. Mopper (1996) argues that local adaptation may be more common among endophagous insects than for externally feeding insects. This may be because endophagous insects feed and live within plant tissue, and then, experience stronger plant mediated selection pressure. In the case of the system studied here (bean / bruchid / parasitoid) bruchids may also be considered as the endophagous hosts, and the parasitic wasp as the exophagous insect. *Zabrotes subfasciatus* seems to be strongly affected by its host plant population (Callejas, 2002) whereas *S. bruchivora* could in turn be adapted to the bruchid population, irrespective of the plant population. The next step should be to test the local adaptation of *S. bruchivora* using not only seeds but also bruchids from the population studied.

This experiment confirms that there is a genetic component of parasitoid performance and that this genetic effect is less expressed in cultivated seeds, which represent an artificial but better host plant. This better seed quality erases the main differences between the wasp

origins. Differences between parasitoid populations in behaviour and performance can also have important implications for the theory and application of biological control. If plant domestication leads to a loss of the variability in wasp behaviour and performance, and these plants are less resistant to the bruchid attack, then farmers should favour host plant variability, in particular wild forms, in a field environment in order to maximize the efficiency of parasitic wasps .

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CONCLUSION and OUTLOOK :

Annexes

The purpose of this study was to investigate the effect of variation in the plant quality on the second and third trophic levels, and to determine the extent to which this variation affects the behavioural and performance traits of herbivores and parasitoids. The relationships between these trophic levels are strong, and modifications on plant quality as a result of domestication have important repercussions on their associated insects. In this case, the humans and the beetles that attack the seeds are competitors. This situation has lead humans to fight against these phytophagous pests. As an example, several methods have been used to control populations of pest species that attack the stored seeds and to prevent the deterioration of these crops (e.g. Baier & Webster, 1992; Abate & Ampofo, 1996; Vasquez-Arista *et al*., 1995).

The chapter on the environmental and genetic components for host use in *Zabrotes subfasciatus* (second trophic level) confirmed the importance of plant quality on the phytophagous insect. Cultivated plants provide the best resource for the insects. Although these results did not show an effect of adaptation of the bruchids to a poor host plant, they revealed that the quality of the offspring is also dependent on the quality of the eggs laid by the females. Moreover, the analysis of laboratory individuals of *Z. subfasciatus*, reared on cultivated seeds, showed differences in fitness parameters among the full-sib families. The sex ratio, the development time and the adult size were influenced by the genotype of the mother. Thus, it seems that a "good" genotype, with higher fecundity and / or shorter larval development time, is able to compensate for a lower host plant quality.

Because of their importance as biocontrol agents, studies on parasitoids are crucial for the better understanding of their complex interactions with the plants and the phytophagous pests that they attack. For example, the knowledge of the spatial distribution of the phytophagous bean pest could improve the efficiency of biological control (Stolk *et al.,* 2001), as well as the impact of the plant species on the parasitoids. Even if the host species is suitable for parasitoid development, the host plant is not always suitable for the good development of the parasitic wasp (Van Huis & De Rooy, 1998). Moreover, competition between two parasitoid species may affect the interaction of these insects with their host. In the case of interspecific competition, females of *Eupelmus vuilleti* have been shown to kill with their ovipositor the eggs and larvae of *Dinarmus basalis* (Leveque *et al.,* 1993).

According to the efficiency as natural enemies on the herbivorous pests, several parasitic wasp species have been studied and used as biological control agents (Van Alebeek, 1996; Eilenberg *et al.,* 2001). Against the pest of the bean seeds, Roomi *et al.* (1997) investigated the life cycle of *Apanteles flavipes*, a gregarious parasitoid of *Bruchus chinensis*, in order to exploit it as a biological control agent on the seeds of *Phaseolus mungo* in Pakistan. In Africa, *Dinarmus basalis* has been largely studied to control the pest *Callosobruchus chinensis* and *C. maculatus* (Islam & Kabir, 1995; Ouedraogo *et al.,* 1996; Sanon *et al.,* 1998). At present, it could be interesting to investigate the efficiency of *S. bruchivora* as an endemic agent of biological control on the *Phaseolus* seeds in America in field conditions.

As in previous experiments (e.g. Benrey *et al*., 1998) results from my study confirm the role of the plant quality on the performance of the third trophic level. A new result is the finding that as a function of their host specificity, the parasitoids *S. bruchivora* and *D. basalis* do not show the same response on wild and cultivated plants. Their parasitism behaviour and variations in their performance differ. These results suggest that these two species do not use the same cues, and do not have the same learning ability which could be a future interesting step in the comparison of these two parasitoid species. One interesting consideration is that these two parasitoid species belong to different families: Braconidae (*S. bruchivora*) and Pteromalidae (*D. basalis*). Although we do not have any evidence, one could speculate that the differences observed in behaviour between these two species are associated to their taxonomy and not to their host specificity. Both are ectoparasitoids, but they do not have the same morphology which could explain some of the differences in host location (differences in the perception of host's cues) and host parasitization (differences in ability of their ovipositor). In order to conclude on whether the differences observed in the behaviour of these two parasitoids are due to their host specificity or to a phylogenetic factor, we would need to examine several species of Braconids and Pteromalids. Mitsunaga & Fujii (1999) investigated

the interspecific interactions on community persistence using two species of beans (*V. angularis, P. vulgaris*), two species of bean weevils (*S. subfasciatus*, *C. chinensis*) and two species of parasitoids (*Heterospilus prosopidis*, Braconidae; *Anisopteromalus calandrae*, Braconidae) that differ in their strength of the density-dependent response to hosts. They concluded that in a multiple-species community, the combination of species itself was more important for community persistence than were the characteristics of the particular species, but did not discuss the influence of the parasitoid phylogeny on the differences found in their results. In 1999, Shimada used almost the same biological system to investigate the effect of resource distribution on the parasitism behaviour (video recording) and the population dynamics. The author found differences between the two parasitoid species according to the host density, but in this case again, the author did not link these differences with a possible greater efficiency of one phylogenic group or the host specificity.

Moreover, the comparison between several populations of the parasitic wasp *S. bruchivora* showed variation in parasitic behaviour and performance, but also variation within a population. It will be interesting to investigate in more detail the genetic origin of these behavioural differences examining the behaviour of hybrids on wild seeds. With a such experiment, I would expect to determine the relative contribution of the both parents in the transmission of the observed differences, and if the transmission is only chromosomic or also cytoplasmic.

Plant domestication can affect the interactions between hosts and parasitoids by providing shelters to the herbivores. Feder (1995) investigated the parasitoid attack on two sympatric races of *Rhagoletis pomonella* (Diptera), one infesting apple (*Malus pumila*) and one infesting hawthorn (*Crataegus* spp.), their ancestral host. According to his results, three factors indicate that *R. pomonella* larvae infesting the derived host apple are able to better

escape parasitism than larvae infesting hawthorn. First, the phenology of the two host plant is different, the seasonal distribution of the parasitoid *Opius lectus* was asynchronous with the development of the host larvae in the apples. Secondly, interspecific interactions are much less common and detrimental for flies infesting apples: heterospecific insects (caterpillars and curculionid weevil) forced fly larvae to feed near the surface of the hawthorn fruit where they were more available for the parasitoids. Thirdly, because the ovipositor of the parasitoid is not long enough and apples are larger than hawthorns, flies feeding in the interior of the apple fruit are better able to escape from parasitism. The bean system presents several characteristics in common with the *Rhagoletis* system. The ancestral host is the wild form of *P. vulgaris*, and the novel host is the cultivated type, with bigger seeds. Because of the use of pesticide on cultivated fields, one could predict that density of the bruchids must be higher on wild seeds, and bean domestication has lead to differential phenology and pod maturity of the cultivated forms (Smartt, 1993). During my work, I only collected parasitoids from wild fields. In order to understand more on the dynamics of parasitoid populations, it seems important to conduct systematic samplings in both wild and cultivated fields. These data could reveal important information on host use in relation to plant availability and plant phenology. In addition, it could provide some information on alternative host plants and hosts used by these parasitoids and / or on their overwintering sites.

Particular interest should be also focused on the great variability on the presence of the bruchid and parasitoid species between successive years. I believe that independently of the localities, several factors must affect the quantity of seeds and insects available. The climatic conditions are of course the main factor, but it appears particularly interesting the dynamic of wild *P. vulgaris* in the field were every two years there is a change in bean density (personal observation and A. Delgado, personal communication).

The last aspect that should be considered in the future is the role of humans in the exchange and transfer of beans between populations (and localities). Bean plants (mainly *P. coccineus* and *P. vulgaris*) have an important economic value worldwide and mainly in Central and Latin America, due to the role they play as part of human diet (Carlderon *et al*., 1992). The selection of the bean is mainly done on the grain quality (acceptability by the consumer, cooking characteristics and nutritive value) and the bean production systems are undergoing transitions, such as in Costa Rica (Bellow *et al*., 1996). The inclusion of new bean varieties can depend mostly on factors associated with the farm size and willingness of the farmers to experiment with the new varieties (Grisley, 1994). In some countries, such as Zambia, low levels of production have been recorded over the years due to the use of local bean varieties. A study was conducted to investigate an efficient production management strategy which indicated that the Brazilian bean variety "Carioca" performed better for the traditional farmers, in combination with fertilizer and insecticide use (Bezuneh, 1992). All along my field work in Mexico, I witnessed the importance of beans in the country's diet, and its presence on rural markets. Several varieties are cultivated there, and it is not rare to find bruchids (and parasitoids) in bean bags. Then, it could be interesting to know if, due to the trade and purchase of beans for their consumption, humans facilitate genetic flow of bruchid (and parasitoids) between localities, and then, participate in the lack of local adaptation found in some of the populations studied in my experiments.

In chapter 3, it was discussed the possibility that the presence of wild forms would allow for the maintenance of variability in behaviour and performance of the parasitoid which in turn would increase its efficiency. Idris & Grafius (1996) arrived to the same conclusions after the investigation of the parasitism of the diamondback moth (*Plutella xylostella*) on wild and cultivated Brassicacae. The presence of wild Brasicaceae in the field could reduce diamondback moth populations. They provide host for *D. insulare*, and so increase the impact of the parasitoid and the success of management programs against *P. xylostella*. Moreover, it is known that polycultures and intercropping increase the effect of predation or parasitism by increasing the diversity of auxiliary insects on phytophagous pests (Coll & Bottrell, 1995; Olubayo & Port, 1997). Thus, if several host plant varieties or species and several phytophagous host species are present in the field, the parasitoids are not faced only with one single factor of selection, such that there may be conflicting forces acting on them. This greater host range should maintain the genetic variability in the parasitoid populations, which should select for a better parasitism efficiency whatever the characteristic of the pest is.

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ANNEXES :

(Things that did not work out the way they were suppose to)

As it is the case for many ecological experiment, during these 3 years, several experiments failed. For some of them the causes were clear and I will discuss them in the section below. For one experiment the cause of failure is still unknown. I believe that addressing these issues is important not only for future work conducted on this system, but also because illustrate the great variation and unpredictiveness that one is faced with while conducting these types of experiment.

I. The study of genetic variation in parasitoid populations:

The geographic structure of a population is a fundamental component of its ecology and evolution that combines both demographic and genetic processes, such as gene flow and migration. When populations are separated in localities far away from each other, they may evolve independently and some degree of genetic variation can be observed (Roderick, 1996).

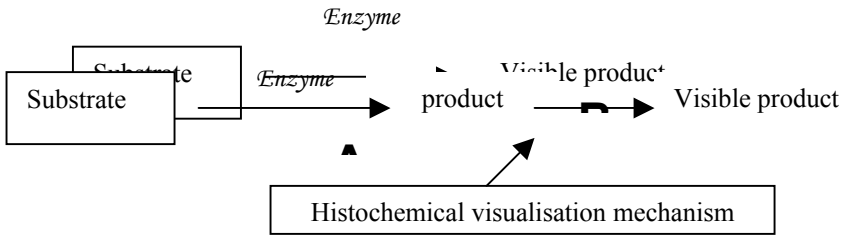
In the relationship between a parasite and its host, or a predator and its prey, the two protagonists are closely linked and generally adapted to each other. This adaptation is sometimes specific to the individuals of the same locality, a process that is know as : "local adaptation" and this process can be limited by gene flow (Van Nouhuys & Via, 1999). Our previous experiments on *Stenocorse bruchivora* (Hymenoptera: Braconidae) and *Dinarmus basalis* (Hymenoptera: Pteromalidae) two parasitoid species of the Mexican bruchid *Zabrotes subfasciatus* (Coleoptera: Bruchidae) showed that these parasitoid species have adopted different strategies to reproduce, and that there exists variation between different populations of *Stenocorse bruchivora* that parasitize hosts offered in wild bean seeds. For *Dinarmus basalis* it was found that development time varied between populations both in relation to the seed type, where it host was offered (wild or cultivated) and also between the parasitoid strains. Due to this variability between the two species of wasps and also within populations, it seemed interesting to investigate the level of gene flow among wasp populations and to determine the spatial scale at which behavioural differentiation occurs.

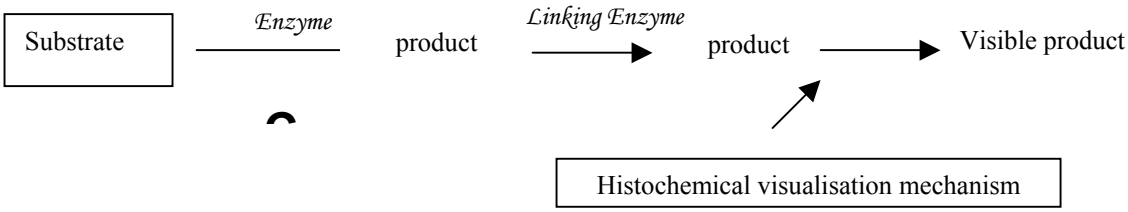
The ability to recognize, understand and identify variation within and between populations is very important in insect ecology, but often difficult to achieve. In the last 30 years, advances in biochemical and molecular techniques such as, electrophoresis, have allowed researchers to explore population differences at the genetic level.

Electrophoresis may be defined as the migration of particles under the influence of an electric field. Enzymes are proteins that catalyse a particular chemical reaction. They are polypeptide chains (chains of amino-acids) with a genetically determined sequence, and catalyse steps in the major metabolic pathways. Isozymes are enzymes that catalyse the same chemical reaction. When isozymes are coded by the same genes but under different allelic forms, they are called allozymes. These chains vary in size and shape and can be positively or negatively charged (or neutral). An electric charge allows to separate the allozymes in an electric field through gels. In these, proteins migrate though several distances due to variation in their charge. This variation in charge is due to amino-acid substitution in the chain which reflects differences in genes. This charge can also vary with the pH of the solution or pH of the gel in which the proteins migrate. At its pHi (isoelectric pH) the charge of the protein is neutral, and the protein does not migrate. The gel as a solid medium, maintains the separation after the electric field is turned off.

The entire process of allozyme electrophoresis can generally be divided in four steps: extraction, separation, staining, and interpretation.

To summarize the method of electrophoresis, a studied sample ("juice" of the entire wasp in this study) is placed on the gel, linked by a buffer solution, to the electrodes of a electrophoresis tank. The proteins migrate under the electric field through the gel. Then they are localized by a colorant (case A) or after a specific catalyse reaction, by its product which can be made visible (case B). In a third case (C) the enzyme is stained to convert the substrate into a product that is not visible but can be converted by a linking enzyme into a second product that can be made visible (Richardson *et al.,* 1986).





According to the gel type used for the migration of the enzymes, there are 4 electrophoresis techniques, the starch gel, the polyacrylamide gels, the agarose gels, the cellulose acetate gels. Each type of gel has its own particular advantages and disadvantages; the choice depends upon the resources available to the researcher and upon the type of project envisaged. The viscosity of the medium is not the same for these four gels types, which allows more effective separation of similarly-charged but different sized molecules : larger molecules are slowed by the difficulty of moving through the small pores in the gel. (Richardson *et al.,* 1986). As an example of the experimental choice of the gel type, Kaufmann (1996) reviewed 87 scientific papers on the use of electrophoresis for the specific study of Formicidae, and calculates that 64 studies used starch gels, 27 used polyacrylamid gels, and just 5 analyses were done with cellulose acetate gels.

Allozymes have been useful in solving many ecological problems such as:

- assessment of population structure such as, effective migration of individuals (Kambhampati *et al.,* 1991; Hasegawa & Yamaguchi, 1995; Banschbach & Herbers, 1996). For social insects, electrophoresis has been usually used to determine the relatedness between workers / queen (the sociogenetic organization) inside and between colonies (Sundström 1993; Aron *et al.,* 1998)
- genetic changes such as, the effects of natural selection on enzymatic systems (Johnson *et al.,* 1969; Keller & Ross, 1993)
- identification and determination of species in taxonomy (Castanera *et al.,* 1983; Pinto *et al.,* 1992); disclosure of new species or detection of sibling species by comparing the pattern of allozyme migration (Narang *et al*. 1989).

Loxdale & Lushai (1998) presented a review of various studies using electrophoresis to investigate several and various biological subjects such as, detection of prey within insect predators (Solomon *et al.,* 1996). This technique was also suitable for detecting endoparasitoids inside their host (e.g. Castrovillo & Stock, 1981).

Allozyme electrophoresis techniques have been used to study a number of organisms. Hebert & Beaton (1989) give a non exhaustive review of some tested plants (*Solidago sp., Betula sp., Polytrichum sp.*), invertebrates such as Protozoa (*Trypanosoma sp.*), Insecta (*Drosophila sp., Aedes sp.*), Crustacea (*Daphnia sp., Leptodiaptomus sp.*), Mollusca (*Dreissena sp.*), Turbellaria (*Mesostoma sp.*), Onychophora (*Peripatus sp.*) and also vertebrates such as, Pisces (*Stizostedium sp.*), and Mammalia (*Pistrellus sp., Eptesicus sp.*).

For bruchid beetles, this technique has allowed to link the differentiation of gene frequencies on several strains of *Callosobruchus maculatus* with several behavioural traits characteristic of each strain (Berg & Mitchell, 1993).

In the case of hymenopteran parasitoids, Powel & Walton (1989) presented a review of 38 studied species (mainly in the Aphidiidae and Trichogrammatidae families), in various areas of study (e.g. genetic drift, taxonomy, detection of parasitism in host). The technique of electrophoresis, in particular on cellulose acetate plates, was used by Atanassova et al. (1998) to investigate the genetic relationship, including reproductive isolation and host adaptation/specificity of five aphid parasitoid species of the genus *Aphidius*. Their results provide evidence of deviation from the Hardy-Weinberg law and for the existence of one or more “morphological-indistinguishable “cryptic” species”.

In this study I used the technique of the cellulose acetate plate with the system provided by Helena Laboratories (PO box 752, Beaumont, Tx 77704-0752, USA) to study the genetic variability between parasitoid's populations. As indicated by Wynne et al. (1991) this method is quick, simple, cheap and has many advantages over starch and polyacrylamid gel electrophoresis. The cellulose acetate plates are bought directly made for use, and have a long shelf live while gels made from starch, acrylamide or agarose have to be made up just prior to

their use. The plates are flexible allowing an easy of handling of the stained gel. The running time is short : between few minutes (15, 30 minutes) to one hour, when starch and acrylamide gels usually run longer, sometimes overnight. This technique has been successful with small invertebrates because it requires small samples. This method allows the detection on the same plate of the cathodal and anodal isozymes. The volume of the staining mixture used is small (+/- 6 ml), as well as the buffer volume, reducing the cost of stains and solutions containing expensive ingredients. One can run two or three acetate plates for different isozymes in the same tank if the buffer and running time are equivalent. Moreover, it allows to reveal two different isozymes by mixing both recipes if these isozymes do not migrate at the same level: for example to stain for both LDH and PGM we have to add 2 drops of lactic acid to a normal PGM stain (Hebert & Beaton, 1989). The isozymes stain more quickly on cellulose acetate plate than on other gel types. Richardson et al. (1986) also indicate that lower voltages are required for cellulose acetate electrophoresis. As a result less sophisticated power packs are needed and the technique is safer to use. The only main disadvantage of this technique is the cost of the plates, and the fact that some enzyme reaction mixtures are highly toxic, e.g. peptidase, which includes snake venom (Richardson et al. 1986; Loxdale & Lushai, 1988).

Materials and Methods: the screening process.

The objective during this screening is the detection of polymorphic enzymes, and the identification of the best recipes of staining for the better interpretation of the gels. I tested 31 enzymes in at least 6 different buffers. For each combination, I tested different voltages (150 to 200 volts) and several running times (30 to 60 minutes), which represent more than 400 tests. All the recipes came from the Helena practical handbook (1989), and Richardson et al. (1986) book.

For this screening I used both parasitoid species: *Stenocorse bruchivora* and *Dinarmus basalis*. The tested insects originated from the second and third generation of a colony composed of several wild wasp

populations collected in the field on infested seeds of *Phaseolus vulgaris*. Even though females are more suitable in the screening of variability and polymorphism, to increase the sample size, both sexes were used.

Cellulose acetate plates (Titan III, Helena laboratories) are immersed and soaked in the selected buffer for several hours (from 4 to 24 h). Prior to their use, the plates are blotted dry between sheets of filter paper. Each wasp insect was individually homogenized in 10 µl of distilled water in a eppendorf vial, and centrifuged for 1 min at 1800 rpm. Since enzymes are soluble in the cytoplasm, a simple rupturing of the cell wall releases them into the solution. Aliquots were applied to the gel surface using a Super Z applicator (Helena laboratories). Loading involves the application of 0.5-1 microlitre of each sample. The applicator allows to have 12 insects on the same cellulose acetate plate. To obtain a better resolution I did 3 or 4 applications at each load zone. Sufficient pressure should be exerted to lightly mark the surface of the plate. It is essential that the position of each slot can be seen after the electrophoresis. This line will be the origin and will allows us to record the relative mobility of the isozyme bands. Once loaded, plates are placed acetate side down on the wicks in the electrophoresis tank, in contact with the buffer via filter paper. Care must be taken in the sense of migration to avoid that enzymes migrate out of the cellulose acetate plate. A microscope slide was use as weight on the acetate plate to ensure complete contact between the plate and the filter paper. Then current is applied at a constant voltage. During the run it is necessary to check that both, voltage and current remain stable. Electrophoresis is ordinarily carried out at room temperature, but in this case I did it in a refrigerator to avoid that the plate would overheat, and to avoid an excess of condensation inside the tank. After electrophoresis, the acetate plates are stained using the appropriate recipe mixed with agar (maintained molten in a drying oven at 60°C). This step must be done once the plates have been removed from the tank, before they dry out. Once the agar has hardened (approximately 1 minute), the plate is placed in the dark at room temperature until protein bands become visible. Temperature

above 25°C can accelerate the staining process. Highly active enzymes may appear within seconds of stain application, while weaker enzymes may take up to an hour. Finalized this step the agar mixture has to be removed by holding the plate under cold running water. Then plates have to be soaked in a tray of water until the water is clear with no colour .

For more details on the method, see the practical handbook from Helena laboratories (1989).

Results:

All the tested combinations are summarized in the Table 1. For each combination I present several gels, with different voltage and time tested. Each number corresponds to a « quality category» of the gel from personal estimation. When an ambiguity between 2 quality categories existed, I indicated the worst.

Legend :

0 : very bad : the gel is illegible. This can be due to lack of staining.

1 : bad : there is a staining on the gel, but the stains cannot be interpreted properly.

2 : correct : the stains are good, but there is no polymorphism.

3 : good : the stains are very good, with polymorphism.

A list of the studied isozymes with their EC number is presented at the end of this chapter. From all these tests (n = 438 gels for more than 2400 insects), I was able to obtain only one polymorphic system for *Stenocorse bruchivora* (the GOT system) which is very interesting to stain and use, and perhaps two polymorphic systems for *Dinarmus basalis* (GOT and IDH systems). Both systems are difficult to study in *D. basalis*.

The different forms and loci of these polymorphic enzymes are summarized in Table 2. The same individuals did not give the same pattern of polymorphism according to the running buffer used and the running time of the migration. For example, for the GOT system for *S. bruchivora*, in 3 cases I obtained the same interpretation (Citrate Phosphate, 1h, 180V; CAAPM, 1h, 200V; TG, 1h, 200V), with one anodal locus with 2 alleles: the first one with a low migration time (a), the second one with a higher migration time (b) for the homozygotic individuals, and a third shape (ab) for the heterozygotic individuals (FIG. 1A). But if one increases the voltage with the Citrate Phosphate buffer (1h, 200 V) or with the Tris Maleate buffer (1h, 200V) it is likely to obtain a very different pattern and more difficult to understand (FIG. 1B and 1C). With the Citrate Phosphate, Tris Maleate and CAAPM buffers, a second locus, cathodal migration, appears and seems also polymorphic in

GOT. With the TG running buffer, the main locus appears alone, and the slow allele migrates to the cathode, the fast allele (b) migrates to the anode and the third band for heterozygote individuals (c) stay on the loading line (FIG 1D).

For *D. basalis*, the GOT system is interesting with its upper locus. It usually appears as a simple band but for some females can be very large. The lower locus is not always present for all samples (FIG 1E). In the IDH system, the first lower locus is no interpretable. The bands are too wide, with a strong coloration. The upper locus is not present in each sample, but in some cases (Citrate Phosphate, 1h, 200 V) it can show polymorphic bands (Fig 1F). It appears that we have a null allele with reduced activity, which in general are not so safe to use in any study (it may lead to an apparent excess of phenotypic homozygotes) and should be avoided even when the specimens do not show enzyme activity (i.e. null homozygotes) cannot be easily explained. Heterozygous individuals have asymmetrical relative band strengths (Richardson et al. 1986). The null homozygote produces no detectable enzyme activity, so that the patterns observed in heterozygotes suggest a subunit structure, and can mimic treatment effects (Richardson *et al.,* 1986).

The few results obtained through the whole screening confirm the poor genetic variation in parasitoids (Graur, 1985; Roderick, 1996), but also on the Hymenopteran group in general (Berkelhamer, 1980; Pamilo *et al.,* 1975). On seven systems, Gonzales-Rodriguez (1997) found only two polymorphic isozymes on the parasitoid *Eupelmus cushmani*, which also parasitises bruchids in *Phaseolus* seeds. In their study, Castrovillo & Stock (1981) detected the presence of the parasitoid *Glypta fumiferanae* (Ichneumonidae) inside its host *Choristoneura occidentalis* (Lepidoptera; Tortricidae). Four of the 15 enzyme systems tested proved useful for detecting the endoparasitoid, but no interpretable polymorphism was found in any parasitoid bands from their samples. Some species and populations of *Aphidius* spp. can have 4 different polymorphic enzymes (Atanassova *et al.,* 1998). In the present study the objective was to evaluate the amount of genetic variation within and among parasitoid populations. One allozyme is not enough to compare different populations. Hence, new and more effective methods as microsatellites must be used to complete this study.

The technique of allozyme electrophoresis is being replaced by new methods of genetic analysis on the DNA in which by the use of molecular markers, the comparison of the genomes offers high resolution and yields character data that also can be examined as sequence divergence. The main problem in using enzymes in genetic analyses, is that most enzymes degrade quickly under even low temperatures. They are not as stable as DNA.

Moreover the method requires to destruction of the sample and the use of fresh or deep frozen material. Another disadvantage of the electrophoresis techniques is that the analysis of allozymes detects only about 30 % of the genetic diversity compared to molecular techniques. This technique however, can still be very useful for other organisms where genetic variability is not so scarce. As exposed in previous paragraphs, the method is cheap and more easily implemented in the laboratory than DNA analyses where more sophisticated equipment is required. In a study on the population structure of *Acanthoscelides obctectus* and *A. obvelatus,* two host bruchid species parasitized by D. basalis and S. bruchivora, Gonzàles-Rodriguez *et al*. (2000) found 5 polymorphic enzymes from a preliminary survey of 15 systems. From a screening of 22 enzyme systems, Müller (1998) found and used six polymorphic loci (PGI, MPI, PEP, GOT I and GOT II, PGM) to establish the genetic population structure of *Gammarus fossarum* (Amphipoda: Gammaridae). Ridgway *et al*. (2001) found and used 5 polymorphic loci after a screening of 22 enzymes on the coral *Pocillopora verrucosa* from 6 coral reefs in South Africa. Mamuris *et al*. (1998) used 14 enzymatic systems coding for a total of 20 loci to differentiate tow red mullet species (*Mullus barbatus* and *M. surmuletus*). The technique on acetate cellulose has also been used for plants as *Allium tricoccum* (Vasseur, 2001), with 13 polymorphic loci to study the genetic variation among three populations, or as *Bdallophyton bambusarum* (GarcYa-Franco *et al*. 1998), with seven polymorphic loci to study the genetic population structure. With this technique Pasquet (1999) studied the genetic variation between wild and cultivated varieties of cowpea (*Vigna unguiculata*). He showed that the wild cowpea exhibits genetic variation perfectly fitted with the existing morphological classification. In this case the electrophoretic allozyme system was successful to confirm the cowpea classification characterized by the breeding systems.

Conclusion:

The lack of variability for our two parasitoid species did not allow us to use the method of the electrophoresis on cellulose acetate plates to investigate the degree of gene flow between the different populations. However, this method is successful for other organisms and with its several positive aspects, should not be always replaced by DNA analyses.

Table 1: Result of the screening of several isozymes tested on 6 buffers. Quality of the gel obtained: 0 : very bad; 1 : bad; 2 : correct; 3 : good (with polymorphism); * : no tested;

Stenocorse bruchivora	ACON	AD	ADH	AK	ALP	AO	ARK	EST	FUM	GOT AAT	G6PDH	GPDH	GPI	HEX	IDH	LDH	MDH	ME	6PGDH	PGM	SOD	XDH	TREHALASE	G3PDH	MPI	LAP	TPI	PEP A	PEP B	PEP C	PEP D
CAAPM	0	0	0	1	0	0	2	2	1	3	2	2	1	1	2	0	2	1	2	2	0	2	1	2	0	2	2	0	*	0	0
TG	0	0	0	0	0	0	2	1	1	3	2	2	1	2	1	0	0	2	2	2	0	1	2	1	1	0	2	2	2	1	0
Citrate Phosphate	1	0	0	2	0	0	2	1	1	3	1	2	0	1	2	0	2	2	2	2	0	1	2	2	0	0	2	1	1	0	1
Tris Maleate	0	*	0	0	0	0	2	0	1	3	0	2	1	1	0	0	2	2	0	2	0	1	1	2	1	0	2	2	2	1	2
D	0	0	0	1	0	0	1	1	1	2	1	2	0	2	1	0	2	*	1	*	0	1	*	*	*	*	*	*	2	0	2
E	0	0	0	0	0	0	1	2	1	2	1	2	0	2	1	0	2	*	2	*	0	1	*	*	*	*	*	2	2	0	0

Dinarmus basalis	ACON	AD	ADH	AK	ALP	AO	ARK	EST	FUM	GOT AAT	G6PDH	GPDH	GPI	HEX	IDH	LDH	MDH	ME	6PGDH	PGM	SOD	XDH	TREHALASE	G3PDH	MPI	LAP	TPI	PEP A	PEP B	PEP C	PEP D
CAAPM	0	0	0	0	0	0	2	2	*	2	1	2	2	1	3	2	1	2	2	2	0	1	1	2	0	2	2	0	*	0	0
TG	0	0	1	0	0	0	2	2	2	2	0	2	2	1	1	2	0	2	2	2	0	0	2	2	*	*	2	2	2	1	1
Citrate Phosphate	0	0	0	2	0	0	2	2	2	3	1	2	1	0	3	2	2	2	2	2	0	1	2	2	0	0	2	1	2	0	2
Tris Maleate	0	*	0	0	0	0	2	0	2	3	0	0	0	0	3	2	2	2	2	2	0	1	1	2	*	0	1	2	2	1	2
D	0	0	0	2	0	0	1	2	2	2	1	2	0	0	2	2	2	2	1	2	0	1	*	*	*	*	*	*	2	0	2
E	0	0	2	0	*	0	1	2	2	2	1	2	0	0	2	2	2	2	2	2	0	2	*	*	*	*	*	2	1	0	2

Table 2:

Species	Enzyme	Conditions	Loci	Alleles	Remarks
S. bruchivora	GOT	Citrate Phosphate 1h 180V	2	2	Dimeric and monomeric
S. bruchivora	GOT	Citrate Phosphate 1h 200V	2	2 or more (?)	New heterozygote forms
S. bruchivora	GOT	CAAPM 1h 200V	2	2	Dimeric and monomeric
S. bruchivora	GOT	Tris Maleate 1h 200V	2	More than 2 (?)	Difficult interpretation
S. bruchivora	GOT	TG 1h 200V	1	2	Dimeric No mark of the cathodal locus
S. bruchivora	GOT	D 1h 150V	1	2	Dimeric The cathodal locus is too pale
S. bruchivora	GOT	E 1h 150V	1	2	Dimeric The cathodal locus is too pale
D. basalis	GOT	Citrate Phosphate 1h 150V	2 ?	In these cases, the upper band seems to have two alleles, while the lower band does not appear for all individuals (null allele?)	
D. basalis	GOT	Citrate Phosphate 1h 180V	2 ?		
D. basalis	GOT	Tris Maleate 1h 180V	2 ?		
D. basalis	IDH	CAAPM 1h 200V	2 ?	The lower locus is not interpretable. The upper locus (null allele ?) is polymorphic.	
D. basalis	IDH	Citrate Phosphate 1h 200V	2 ?		

List of the tested isozymes:

6PGD = 6PGDH : 6-Phosphogluconate dehydrogenase	1.1.1.44
ACON = ACOH : Aconitase Hydratase	4.2.1.3
AD : Aldehyde Dehydrogenase	?????
ADH : Alcohol dehydrogenase	1.1.1.1
AK : Adenylate Kinase	2.7.4.3
ALP : Alkaline Phosphatase	3.1.3.1
AO : Aldehyde Oxidase	1.2.3.1
ARK : Arginine Kinase	2.7.3.3
EST : (Carboxyl) Esterase	3.1.1.1
FUM : Fumarate Hydratase	4.2.1.2
GPDH = αGPD : Glyceraol-3-Phosphate Dehydrogenase	1.1.1.8
G3PDH : Glyceraldehyde-3-Phosphate dehydrogenase	1.2.1.12
G6PD = G6PDH : Glucose-6-Phosphate Dehydrogenase	1.1.1.49
GOT = AAT : Aspartate Amino Transferase	2.6.1.1
GPI = PGI : Glucose-6-Phosphate Isomerase	5.3.1.9
HEX = HK : Hexokinase	2.7.1.1
IDH : Isocitrate Dehydrogenase	1.1.1.42
LAP : Leucine Aminopeptidase	3.4.11.1
LDH : Lactate Dehydrogenase	1.1.1.27
MDH : malate Dehydrogenase	1.1.1.37
ME : Malic Enzyme = Malate Dehydrogenase $NADP^+$	1.1.1.40
MPI : Mannose-6-Phosphate Isomerase	5.3.1.8
PEP A : Peptidase valine-leucine	3.4.11.* or 3.4.13.*
PEP B : Peptidase Leucine-glycine-glycine	3.4.11.* or 3.4.13.*
PEP C : Peptidase Lysine-leucine	3.4.11.* or 3.4.13.*
PEP D : Peptidase Phenylalanine-proline	3.4.11.* or 3.4.13.*
PGM : Phosphoglucomutase	5.4.2.2 or 2.7.5.1
SOD : Superoxide Dismutase	1.15.1.1
TPI : Triose-Phosphate Isomerase	5.3.1.1
α, α-Trehalase :	3.2.1.28
XDH : Xanthine Dehydrogenase	1.1.1.204

Running buffers:

CAAPM : pH: 7
TG (Tris Glycine): pH: 8.5
Citrate Phosphate : pH: 6.4
Tris Maleate : pH:7.8
D (Tris-EDTA-Borate- MgCl2) : pH: 7.8
E (Tris-EDTA-Borate- MgCl2) : pH: 8.9

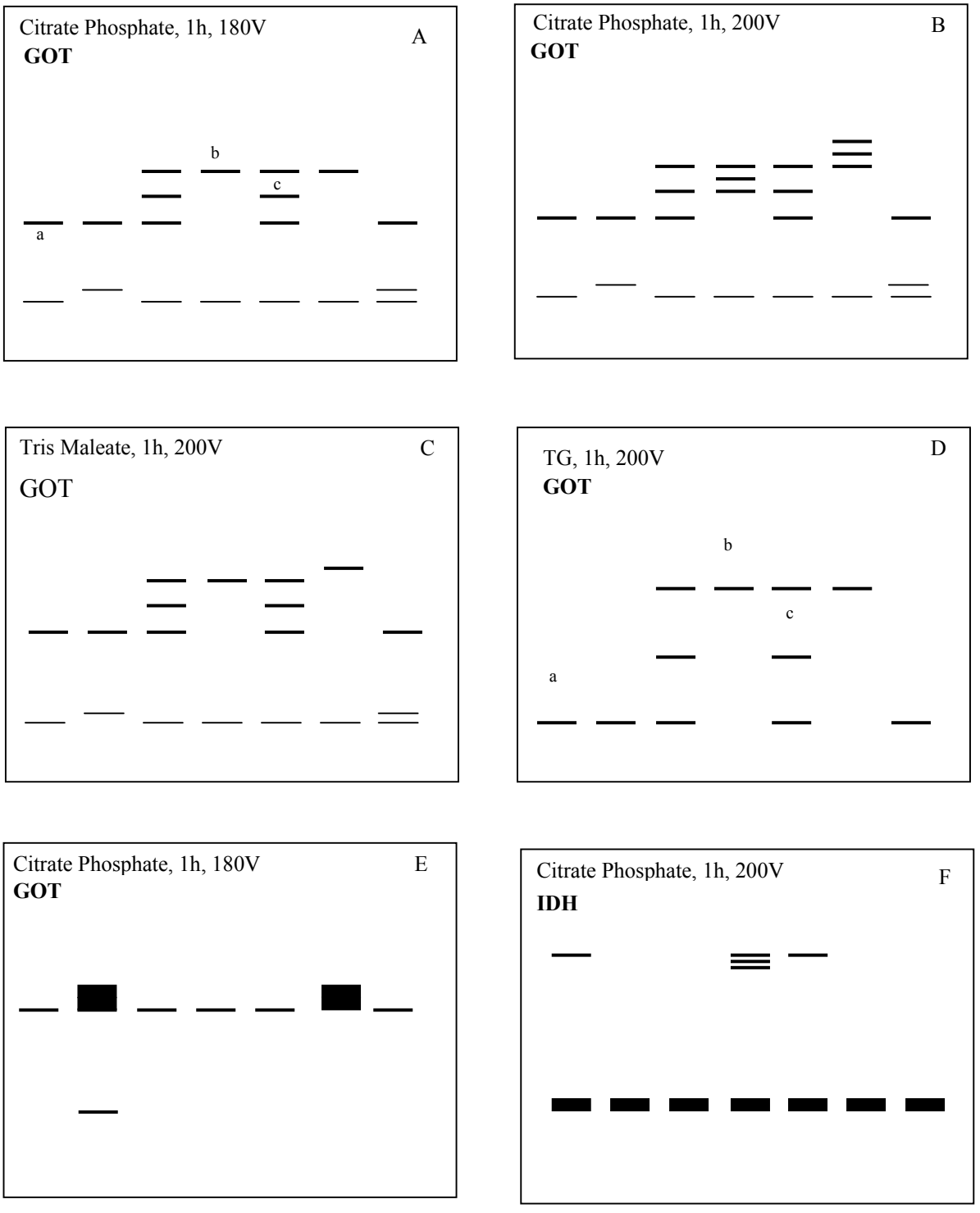


Figure 1: Polymorphic patterns found in the screnning of *Stenocorse bruchivora* (A, B, C, D) and *Dinarmus basalis* (E, F). Small letters refer to the running time of alleles and the pattern of the sample: a: slow homozygote; b: fast homozygote; c: heterozygote.

II. Local adaptation and hybrid study:

II a: Local adaptation:

This experiment was conducted during the first field season. The objective was to investigate if parasitic wasps are adapted, and develop better on hosts reared on seeds from their population of origin. Through transplant experiment, I studied the behaviour and performance of individuals from several populations of *D. basalis* and *S. bruchivora* tested on wild seeds, in order to reveal a possible local adaptation process. I planned to study the fitness of the G2 insects (sex, size, development time).

Material and methods :

I used wild and cultivated seeds of *Phaseolus vulgaris* (Leguminosae), one laboratory colony of *Zabrotes subfasciatus* (Coleoptera: Bruchidae), and the two parasitoid species, *Dinarmus basalis*, wild insects from the populations of Tepoztlan, Malinalco, Atila, and a laboratory colony, and *Stenocorse bruchivora*, wild insects from the populations of Tepoztlan and Atila.

Due to phenological differences among the populations, the pods were not mature at the same time. In Malinalco and Atila, the pods matured faster than in Tepoztlan. From a fourth population, Ahuehueyo (close to the population of Atila) that I had planned also to use in this study, no insects emerged from the seeds , even though they appeared to be infested. I suspect a contamination by pesticide from a nearby sugar cane field.

D. basalis: Infested seeds from Malinalco were collected the 29.01.00 and some parasitoid individuals emerged from these, however they did not produce the subsequent generation. Then for this experiment I used males originated from this population but from the previous year, and reared in the laboratory on seeds from the same population. Seeds from Tepoztlan were collected the 26.01.00. Parasitoids emerged from these seeds but only 9 males were obtained in the subsequent generation. The wild Atila population was started with 10 females, and 3 males (collected 05.03.00). I also used males and females from a laboratory colony.

***Stenocorse bruchivora*: The Tepoztlan population, was started with 24 females and 14 males (collected on the 26.01.00 ; 10.02.00 ; 05.03.00); and 49 females and 35 males started the population of Atila (collected the 05.03.00).**

After the collection of wild pods, I waited for the emergence of the wild parasitoids (Go). They were placed in a aquarium with food and cultivated seeds of *P. vulgaris* (black bean variety), infested with *Zabrotes subfasciatus* larvae of 18 days old. After 15 days, each seed was isolated in a plastic container. The use of cultivated seeds was to avoid a maternal effect of the origin of the mother parasitoid from wild seeds during the test. Each day, I took out the new G1 virgin insects when they emerged. After 3 days, insects were coupled, and when the females were 6 days old, each couple was placed in plastic box with 10 wild seeds to obtain the G2 generation. Each wild seed was infested by a single *Zabrotes* larva of 18 days old. After 24 hours, the couple was moved to a second plastic box with several infested black beans during another 24hours. This step was done to maintain the genotype of the couple and start populations. Both insects were placed in alcohol for future measurements.

For some couples, before the oviposition on black bean infested seeds, infested wild seeds from a different population than the previous one were offered to the couple. This was done to compare the performance and preference of females of each wasp population after a previous parasitism experience.

The experiment was initiated using *D. basalis*, the first species that appeared in the field. But after several weeks, this species was no longer found and was substituted for *S. bruchivora*. Due to the lack of seeds and the emergence problems of the insects, we did not tested all the couples. These insects were used to start and

SPECIES	COUPLES F1 female X male	N°	SEEDS USED	G2 (m = male)
Dinarmus basalis	Laboratory X Tepoztlan	6	2 Ah; 3 M; 4 T;	1m
	Laboratory X Malinalco	6	3 Ah; 4 M; 4 T;	0
	Laboratory X Laboratory	15	6 At; 5 Ah; 6 M; 7 T;	0
	Atila X Atila	12	1 Ah; 5 At; 3 M; 6 T;	1m
	Atila X Laboratory	7	1 Ah; 2 At; 2 M; 2 T;	0
Stenocorse bruchivora	Atila X Atila	26	6 Ah; 8 At; 9 M; 9 T;	12m
	Atila X Tepoztlan	21	7 Ah; 5 At; 7 M; 5 T;	7m
	Tepoztlan X Tepoztlan	20	2 Ah; 6 At; 7 M; 7 T;	1m
	Tepoztlan X Atila	10	1 Ah; 4 At; 3 M; 3 T;	0

increase new colonies on black beans.

Table 9: This table shows the number of tested couples, and the number of G2 individuals obtained on wild seeds: there were only males. The different letters indicate the locality of origin of the seeds where hosts were offered to the female parasitoids: Ah : Ahuehueyo ; At : Atila ; M : Malinalco ; T : Tepoztlan.

I did not have enough insects in the G2 generation to make a statistical analysis of the fitness parameters for these insects. In addition, those insects that emerged were very small, most probably due to an unsuitable host stage. The bruchid hosts from these seeds emerged later than normally which indicates that their larvae were too young when exposed to the parasitoids.

The few results obtained in this study indicated that females of *Stenocorse bruchivora* from the population of Atila were more successful in parasitizing the hosts, or perhaps less sensitive to a change in conditions (from a natural environment to laboratory environment) than females originated from the population of Tepoztlan.

The lack of individuals for the G2 can be due to several factors :

- A short time for the oviposition period : we allowed the female parasitoid to lay eggs during 24 hours. Nishimura (1993) reported that *D. basalis* is able to lay 15 eggs per day. This period should be enough for the 10 seeds offered. This length of time was chosen to avoid wasps laying eggs on all hosts offered which could confound any potential preference for a particular seed type.

- A lack of oogenesis stimulation : Gauthier et al. (1997) placed the newly emerged parasitoids in Petri dishes in the presence of seeds infested by 24h-old-host, to stimulate the oogenesis. I do not believe that this was the cause of lack of oviposition in my experiment. If that would be the case, the couples that were 2 or 3 times in contact with wild seeds before being exposed to the black beans, should have laid more eggs. The females were dissected and showed the presence of mature ovocytes.

- Are females inseminated ? If only males had emerged from this experiment, we could be sure that the females were not inseminated. But this is not likely with the full sib couples. Firstly, because G2 females emerged from the black beans (see experiment with hybrids). Secondly, in some cases, G1 males and females emerged at the same time from the same isolated seed. In this case, I kept these insects together as one couple for a period of 6 days before the test and would be unlikely that during this time mating would have not taken place.

- Sex allocation : In social insects such as, the ants, the Constant Male Hypothesis (CMH) predicts that if there is competition among related males for access to mates, colonies should produce all males up to some threshold and then, if resources allow investment beyond this threshold, they produce only females (Franck, 1987). It is possible that parasitic wasps could do the same and preferred to lay haploid eggs before diploid eggs. This have to be test in a behavioural experiment.

In conclusion the lack of the G2 offspring in these experiment was mainly due to the wrong host larval stage, so that females did not parasitize these hosts, except to oviposit some males that may require less nutrients to develop.

II b: Hybrids study:

I used newly emerged wasps from isolated black beans to test the possibility of viable hybrids between our different populations. I crossed virgin females that originated from laboratory colony or wild populations with males chosen in the other populations. After 24h, I allowed the females parasitoids to oviposit on cultivated seeds infested with several 18 days-old *Z. subfasciatus* larvae. But here again there were not enough female individuals that emerged from black beans. Thus, I could not evaluate the existence of genetic differentiation in the behaviour and fitness components of the parasitoid populations.

SPECIES	COUPLES G1 female X male	HYBRIDS G2
Dinarmus basalis	Laboratory X Tepoztlan	No
	Laboratory X Malinalco	Yes
	Laboratory X Laboratory	***
	Atila X Atila	***
	Atila X Laboratory	Yes
Stenocorse bruchivora	Atila X Atila	***
	Atila X Tepoztlan	Yes (only one time)
	Tepoztlan X Tepoztlan	***
	Tepoztlan X Atila	No

Table 10: Name and sample size of the genetic crosses done for the hybrid study. The column of hybrids G2 indicate if diploid females (true hybrids) were obtained.

III. Isolines of parasitic wasps :

I conducted this experiment to investigate the role of the genotype of the female parasitoid on its parasitism behaviour and performance of her offspring.

Wild females of *Stenocorse bruchivora* were isolated with one male, and placed individually in a plastic box 1) with several wild *Phaseolus vulgaris* seeds infested each one with one or two 21 days old *Zabrotes* larvae, or 2) with black bean seeds infested by two or three 18 days old *Zabrotes* larvae. During 5 days the wasps were allowed to parasitize the seeds. At the emergence of the new generation, I isolated almost all the G1 females with one of their brothers to parasitize new infested seeds to obtain the G2 generation.

I did not obtain enough individuals from the wild seeds to start an isoline (Table 3). From the black bean seeds, I got many wasps (G2). Due to the good quality of both seeds and larval stage, the sex ratio was strongly female biased. Unfortunately a mite attack killed almost all the individuals from the G2 generation. Few wasps emerged and these wasps did not survive more than two days, not enough to parasitize new infested seeds (Table 3).

Table 11. number and sex (m: male, f: female) of the G1 and G2 offspring that emerged for each tested couples used to start the isolines (G0)

Wild seeds		Cultivated seeds (black beans)		
G0	G1	G0	G1	G2 (alive)
Atila 1	3f + 1m	Atila 1	17m	
Atila 2	4f + 2m	Atila 2	2m + 14f	1f
Atila 3	7m	Atila 3	1m + 9f	2f
Atila 4	2m	Atila 4	3m + 11f	1f
Malin 1	0	Atila 5	1m +1f	3f
Malin 2	0	Atila 6	5m + 8f	
Malin 3	0	Malin 1	2m + 7f	
		Malin 2	5m + 3f	1f
		Malin 3	3m + 17f	
		Malin 4	1m + 4f	
		Malin 5	5f	
		Malin 6	2f	
		Tepozt 1	5m + 7f	
		Tepozt 2	4m + 3f	
		Tepozt 3	2m + 5f	3f
		Tepozt 4	6m + 9f	2f
		Tepozt 5	3m + 4f	2f
		Tepozt 6	1m + 5f	3f

In summary, this experiment failed and I did not obtain enough wasps, and isolines to test and compare them. But this experiment showed that one female *Stenocorse bruchivora* is able to parasitize 20 hosts in 5 days on cultivated seeds.

VI. Isolines of *Zabrotes subfasciatus* :

This experiment was conducted to study the genetic component in the variability on the behaviour and the performance of *Zabrotes subfasciatus*. Seeds were collected from 4 wild populations of *Phaseolus vulgaris*, the main host plant of *Z. subfasciatus,* and taken to the laboratory to wait for the emergence of the bruchids. From one of the four populations (Tepoztlan), I did not obtain *Z. subfasciatus*. From the 3 other populations (Atila, Ahuehueyo and Malinalco) adults were divided in two groups: one group was placed on wild seeds to maintain a colony of each different population, and the other group was used to start the isolines. Each G0 female was isolated in a plastic box with wild seeds. I used more than 90 females. Due to the very low quantity of male *Zabrotes* found in the field, many females were virgin and did not lay eggs. I obtained 10 boxes (10 lineages) with eggs from beetles originated from Ayueyueyo, 10 from Atila and 7 from Malinalco.

After 3 weeks, I collected the newly emerged offspring of each female to isolate them. All the offspring were females. Therefore, to continue with the isoline and mate the females, I used males from a laboratory colony (one male to mate all the females from the same mother). Subsequently, I placed each G1 female in a box with wild seeds and waited for the presence of eggs. After 2 weeks, the females had not laid eggs. The females were then placed with a new laboratory male *Zabrotes* in a gelatine capsule during 2 days. Fresh infested wilds seeds of *P. vulgaris* were offered to the females, but females did not lay eggs.

Because these wild seeds were originated from the same field collection than other successful experiments with parasitic wasps, I disregarded the possibility of the presence of pesticide on the seeds. The origin of the problem seems to be directly linked to the bruchid females and still not explained.

One unexpected observation was that that year the sex ratio of the bruchid was strongly female biased in the field and in the laboratory. Other colleagues working with *Zabrotes subfasciatus* also reported the same problem (Callejas A. & Flores Martinez A. personal communication).

V. The negative effect of Mites:

In several occasions, the insect colonies suffered from mite infestations that killed almost all the individuals. This mites were determined as *Pyemotes* spp. (Acari: Pyemotidae) (Photo 1 & 2).

Pyemotes mites are ectoparasites of many insect species linked with stored grains and other crops such as, wheat, barley, beans, peas, straw, cottonseed, tobacco, hay, grass and broom corn (Rycroft & Kennedy, 1981). Grob *et al*. (1998) presented a list of the main insect genera attacked by piemontid mites. Feeding females are between 0.2 to 0.3 mm, colorless, or lightly yellow. There is a strong sexual dimorphism, males are smaller and do not have pseudostigmatal organs. Fertile females search suitable hosts, they inject their poisonous saliva which paralyses the host. Female mites suck the host hemolymph, and the size of their abdomen increases and can exceed 1 mm. Inside the abdomen, the new mite generation grow, and few days later are born and ready to copulate (vivipary). Under optimal conditions, one female can produce 200 offspring. Male mites live upon the gravid female and copulate with the new females at their emergence. The females are very mobile, and die after 36 h if they have not found any host. The gravid females can over winter, the first offspring appear in summer (25- 28 °C) (Grob *et al*., 1998).

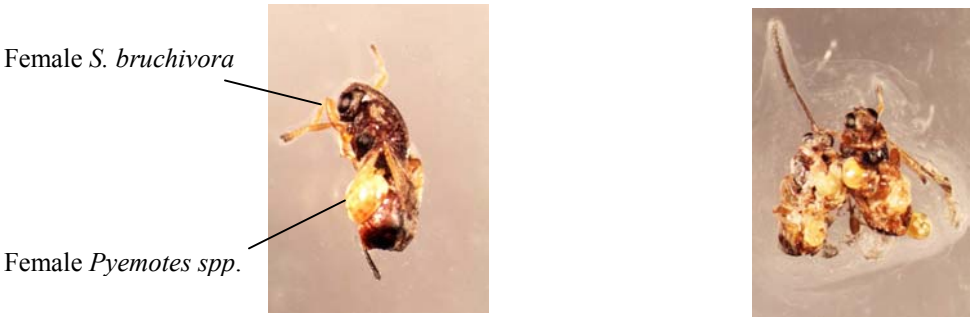
The mites may infest incidental hosts such as horses (Kunkle & Greiner, 1982) and humans (Grob *et al*., 1998; Uenotsuchi *et al*., 2000). Human infestation occurs only accidentally and temporally: the mites reside briefly on humans and cause dermatoses. The symptoms appear acutely, usually 12-16h after the mite attack. The dermatitis is confined to arms and trunks. Bites produce an intensely itching urticarial wheal capped by pin-point vesicles, and pruritic oedematous erythematous lesions (Grob *et al*., 1998; Uenotsuchi *et al.,* 2000). The weal usually subsides after 48-72h if not excoriated, but may persist for several days if scratched (Letchford et al., 1994; in Uenotsuchi *et al*., 2000). In some case affected patients have a systemic symptoms such as fever, diarrhea, anorexia and malaise (Betz *et al.*, 1982). Warehouse workers using infested decorative wheat, or workers who had unloaded

brome seed bags or husk rice bags, can be attacked. On brome seed bags, the densities of mites ranged from 0.7 to 5.6 mites per cm2 (Walker & Landis, 1994).

To prevent infestation by pyemotid mites in their experiment, Hetz & Johnson (1988), proposed the use on paper towel impregnated with 1% Kelthane. To protect their colonies of *Phoracantha semipunctata* on which *P. tritici* caused paralysis and reduced longevity, Hanks *et al*. (1992) applied dust of sulfur to the logs containing pupating beetles. The sulfur may physically impede the dispersal of immature mites by adhering to the cuticle. In case of human attack, fumigation of the storage facilities with the insecticide methylbromide and carbamyl provides successful eradication of the mite and their hosts (Grob *et al*., 1998; Uenotsuchi *et al*., 2000). Insect repellents containing DEET, sulphur, or gammabenzene hexachloride ointment are effective in preventing mite bites (Walker & Landis, 1994; Uenotsuchi *et al*., 2000). Treatment with oral antihistamines and topical corticosteroid make skin lesions disappear (Uenotsuchi *et al*., 2000).

Pyemotes spp. is a serious threat to the good progress of entomological experiments. Matthews (2000) observed mortality of *Psenulus interstitialis* (Hymenoptera) and Marei (1992) found 80% of mortality in the laboratory rearing larvae of *Chrysopa carnea* (Neuroptera) due to pyemotid mite. In my experiment, I lost almost 90 % of the individuals from the new parasitoid generation which resulted in a very small number of tested insects in some experiments, and in some cases experiments could not be conducted. In the tritrophic system studied in this project, *Pyemotes spp.* were observed attacking the host *Zabrotes subfasciatus* (Coleoptera) and both parasitoid species *S. bruchivora* and *D. basalis* (Hymenoptera). Personal observations indicated that from these three species, *S. bruchivora* is the most sensitive to the mite attacks. A whole colony can be lost in one generation, whereas *D. basalis* and *Z. subfasciatus* may survive with a low infestation rate. Because this project involves insects, the use of pesticide (Spomil © 25% of bromopropylate, Maag) on beans was unsuccessful (mouldy bean, and dead parasitoids). The best method consisted in removing all the colonies, disinfecting the incubators and initiating new colonies with cleaned insects, checked individually with a binocular microscope. This method although effective was incredibly time-consuming.

Mites can be so effective in eliminating insect colonies, that some species such as, *Pyemotes tritici* have been used as biological control agents against the Mediterranean flour moth, *Anagasta kueniella* (Lepidoptera). With the release of 80 females of *P. tritici* 10 days after the eggs were laid, a complete control and sometimes complete destruction of a cohort of 400 individuals of *A. kuehniella* was obtained (Hoschele & Tanigoshi L.K., 1993).



Photos 1 & 2: individuals of *Stenocorse bruchivora* parasitized by physogastric females of *Pyemotes* spp. (Photo: Y. Borcard).

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