

ANTHROPOGENIC EFFECTS ON POPULATION GENETICS OF PHYTOPHAGOUS INSECTS ASSOCIATED WITH DOMESTICATED PLANTS

Nadir Alvarez,^{1,2,3} Martine Hossaert-McKey,^{2,4} Gwendal Restoux,^{2,5,6} Alfonso Delgado-Salinas,^{7,8} and Betty Benrey^{1,9}

¹Laboratoire d'Entomologie Evolutive, Université de Neuchâtel, 11 rue Emile-Argand, Case Postale 158, CH-2009 Neuchâtel, Switzerland

²Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175, CNRS, 1919 route de Mende, F-34293 Montpellier Cedex 5, France

³E-mail: nadir.alvarez@unine.ch

⁴E-mail: martine.hossaert@cefe.cnrs.fr

⁵Unité de Recherches Forestières Méditerranéennes, UR 629, INRA, Site Agroparc, Domaine Saint Paul, F-84914 Avignon Cedex 9, France

⁶E-mail: gwendal.restoux@avignon.inra.fr

⁷Departamento de Botánica, Instituto de Biología, UNAM, Apartado Postal 70-233 Coyoacan 04510 México, D.F., Mexico

⁸E-mail: adelgado@ibiologia.unam.mx

⁹E-mail: betty.benrey@unine.ch

The hypothesis of isolation by distance (IBD) predicts that genetic differentiation between populations increases with geographic distance. However, gene flow is governed by numerous factors and the correlation between genetic differentiation and geographic distance is never simply linear. In this study, we analyze the interaction between the effects of geographic distance and of wild or domesticated status of the host plant on genetic differentiation in the bean beetle *Acanthoscelides obvelatus*. Geographic distance explained most of the among-population genetic differentiation. However, IBD varied depending on the kind of population pairs for which the correlation between genetic differentiation and geographic distance was examined. Whereas pairs of beetle populations associated with wild beans showed significant IBD ($P < 10^{-4}$), no IBD was found when pairs of beetle populations on domesticated beans were examined ($P = 0.2992$). This latter result can be explained by long-distance migrations of beetles on domesticated plants resulting from human exchanges of bean seeds. Beetle populations associated with wild beans were also significantly more likely than those on domesticated plants to contain rare alleles. However, at the population level, beetles on cultivated beans were similar in allelic richness to those on wild beans. This similarity in allelic richness combined with differences in other aspects of the genetic diversity (i.e., IBD, allelic diversity) is compatible with strongly contrasting effects of migration and drift. This novel indirect effect of human actions on gene flow of a serious pest of a domesticated plant has important implications for the spread of new adaptations such as resistance to pesticides.

KEY WORDS: *Acanthoscelides obvelatus*, Bruchidae, drift, host dispersal, isolation by distance, migration.

The balance among mutation, drift, selection, and migration drives the genetic structure of populations. Whereas local selection and drift promote genetic differentiation, gene flow acts to homogenize it. Gene flow is governed by numerous factors, among them the dispersal capability of individuals and the frequency of extinction and recolonization events (Slatkin 1987; Whitlock and McCauley 1990; Rousset 1997). The most intuitive pattern of variation in gene flow is its global decrease with geographic distance. As proposed by Wright (1943), the model of genetic isolation by distance (IBD) predicts that genetic differentiation increases with geographic distance. In their review, Peterson and Denno (1998) showed that slopes of IBD decrease with increasing mobility of the species considered. They thus suggested that highly mobile species, such as most insect pests, are characterized by high levels of gene flow compared to species occurring in natural habitats, and postulated that nonequilibrium conditions are frequent in such mobile species. However, in the majority of studies, they observed that there was considerable scatter in the IBD relationship, and attributed it to populations or individuals either more differentiated than expected by distance alone, or less differentiated than expected by distance alone, because of variation in genetic drift or in other processes associated with extinction and recolonization.

Factors that mold the genetic structure of phytophagous insect populations have been broadly studied in the last decade—by means of molecular tools—in natural populations (e.g., Costa and Ross 1994; Keyghobadi et al. 1999; Peterson et al. 2001; Brouat et al. 2003). Other studies have considered the genetic structure of populations of pest species in agricultural environments or managed woodlands (e.g., Congdon et al. 1997; Sembene and Delobel 1998; Kerdelhué et al. 2002; Nahrung and Allen 2003). Herbivorous insects that achieve pest status usually continue to exist in natural habitats alongside managed ones. Those in managed habitats may, however, be directly dispersed by humans when they are transported along with domesticated plants. This anthropogenic impact on dispersal should generate strong differences in population structure between conspecific insects in natural and managed habitats, and also affect geographic patterns of genetic differentiation. The study of this differentiation promises to be both conceptually interesting, in that it makes use of the anthropogenic effects on dispersal as a natural experiment, and practically useful, in that it may help predict insect evolutionary responses to pest control techniques. However, comparison of population structure on wild and domesticated host plants, in phytophagous insects that occur on both host types, is a largely unexplored theme. Until now, only two studies have compared the populations of pest species both on domesticated host plants and on their wild counterparts. Both studies focused on whether populations originating from domesticated plants and those from wild relatives were genetically differentiated (i.e., differed in al-

lele frequencies). The first, on the aphid *Brevicoryne brassicae*, showed such differentiation (Ruiz-Montoya et al. 2003) whereas the second, on the bruchid beetle *Acanthoscelides obtectus*, found no such structuring (Gonzalez-Rodriguez et al. 2000). However, focusing exclusively on differentiation gives a very partial view of how population structure of phytophagous insects is affected by domestication of a host plant.

Here, we explore one step further, by investigating whether differences in migration and demographic stochasticity have led to different patterns in gene flow and diversities between populations on domesticated and on wild plants. We examine the effect of the kind of host plant (wild vs. domesticated) on IBD and patterns of genetic diversity within and among populations. We use a species, the bean beetle *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae), whose intrinsic dispersal capability is augmented by human-mediated migrations. Individuals of this beetle seem unable to disperse more than 2 km by flying (see Jarry 1984), whereas they can travel several hundreds of kilometers through human-mediated migrations of bean seeds (C. D. Johnson, pers. comm. 2004; see also Alvarez et al. 2005a). This difference leads us to expect that IBD should be stronger among pairs of populations feeding on wild host plants than among pairs of populations developing on domesticated host plants.

Material and Methods

SAMPLING SITES

Acanthoscelides obvelatus individuals were obtained from bean seeds collected from 19 Mexican populations, between December 2001 and March 2002 (Table 1; Fig. 1a). Mexico is an ideal place to perform this type of study, because it is one of the centers of domestication of *Phaseolus* beans, where domesticated and wild populations coexist in sympatry. Among the sampled populations, 13 were from wild populations of bean species of the *Phaseolus vulgaris* group (see Delgado-Salinas et al. 1999), *P. coccineus* and *P. vulgaris*. The six other populations were from domesticated populations of *P. vulgaris*. In four of the 13 sites from wild populations, both bean species were present and used as host plants. To test whether the host plant species (*P. coccineus* [c] or *P. vulgaris* [v]) could drive differentiation in the beetle *A. obvelatus*, we first considered the sites in which both bean species were present, as being composed of two different “subgroups” (c or v), according to the bean species on which a given beetle was sampled.

MICROSATELLITE ANALYSIS

Total genomic DNA of 16–24 individuals per population (or “subgroup”) was extracted using DNeasy™ kit (Qiagen, Hilden, Germany). González-Rodríguez et al. (2000) tried to assess the genetic structure of *A. obvelatus* using allozyme markers but

Table 1. List of sampled sites. The kind of bean is referred to as “d” for domesticated and as “w” for wild. Populations issued from domesticated beans are noted with a “*” besides the code. In sites in which both host plant species were present, the number of individuals sampled on each of the species is given separately (*P. coccineus* [“c”] and on *P. vulgaris* [“v”]).

Code	Site name	Bean species	Kind of bean population	Longitude (W)	Latitude (N)	Altitude (m)	Number of individuals analyzed
COP*	Copándaro—campo	<i>P. vulgaris</i>	d	101°46'27.3''	19°26'36.2''	2120	24
COP	Copándaro	<i>P. coccineus</i> , <i>P. vulgaris</i>	w	101°46'11.8''	19°26'26.1''	2117	46 (24 [c], 22 [v])
ERO	Erongaricuaro	<i>P. vulgaris</i>	w	101°42'32.8''	19°35'56.3''	2072	24
HUI	Huitzilac	<i>P. coccineus</i>	w	99°16'23.3''	19°01'24.0''	2544	21
MAL	Malinalco	<i>P. coccineus</i> , <i>P. vulgaris</i>	w	99°30'08.9''	18°57'13.2''	1935	45 (23 [c], 22 [v])
NAP*	Napizarro	<i>P. vulgaris</i>	d	101°41'33.6''	19°35'50.5''	2060	21
SAG	San Andrés de los Gabeles	<i>P. vulgaris</i>	w	99°57'01.5''	19°02'19.5''	2280	16
SBO	San Bartolo	<i>P. vulgaris</i>	w	100°03'20.8''	19°14'31.7''	2320	24
SFP*	San Francisco Peribán	<i>P. vulgaris</i>	d	102°24'28.4''	19°32'32.4''	1800	22
SIC	San Isidro cerca Coeneo	<i>P. vulgaris</i>	w	101°34'23.9''	19°50'56.6''	2040	16
SJS*	San Jose de los Laureles—campo	<i>P. vulgaris</i>	d	98°59'35.0''	18°58'48.1''	1800	23
SJS	San Jose de los Laureles	<i>P. coccineus</i> , <i>P. vulgaris</i>	w	99°00'05.0''	18°58'49.7''	1855	37 (20 [c], 17 [v])
SLO*	San Lorenzo	<i>P. vulgaris</i>	d	102°06'43.4''	19°31'33.3''	2125	21
SUL	Sultepec	<i>P. vulgaris</i>	w	99°59'20.9''	18°51'07.8''	2164	23
TEM	Temascaltepec	<i>P. coccineus</i> , <i>P. vulgaris</i>	w	100°02'44.2''	19°02'35.9''	1734	45 [23 [c], 22 [v])
TEP	Tepoztlán	<i>P. vulgaris</i>	w	99°07'15.7''	18°59'36.3''	1931	16
TLA	Tlalpan	<i>P. coccineus</i>	w	99°12'04.3''	19°17'50.3''	2403	23
TZI*	Tzintzuntzan	<i>P. vulgaris</i>	d	101°34'41.5''	19°37'43.9''	1980	24
VDB	Valle de Bravo	<i>P. vulgaris</i>	w	100°07'05.1''	19°13'56.8''	1918	23

these were not variable enough to establish differences in allele frequencies among populations. Our study used highly variable microsatellite markers now available for this species: three previously developed microsatellite loci AcobC09, AcobD04, and AcobD06 (Alvarez et al. 2003) and three new loci AcobF11, AcobG09, and AcobH01 (Table 2), developed according to the same methods described in Alvarez et al. (2003). PCR amplifications were performed, following the standard protocol of the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany), in a final volume of 10 μ l, which contained about 5 ng of extracted DNA, 5 μ l of 2 \times Multiplex PCR Master Mix (1 \times at final concentration), 1 μ l of 5 \times Q-Solution (Qiagen, Hilden, Germany) (0.5 \times at final concentration), and 0.2 μ M of each multiplexed primer. Products of the PCR reactions were then analyzed on ABI Prism 310 and 3100 sequencers. Genotypes were analyzed using Genscan 3.1.2 and Genotyper 2.5 softwares (Applied Biosystems, Foster City,

CA). Exact sizes of alleles were sorted by size values and plotted in Excel, and further corrected to round integer sizes.

GENETIC STRUCTURE AND DIVERSITY AMONG POPULATIONS

Genotypic disequilibrium between the different pairs of loci, departure from Hardy–Weinberg equilibrium for all loci, and *F*-statistics within and between beetle populations (using an infinite alleles model [IAM]) were tested or calculated using Genepop 3.4 (Raymond and Rousset 1995). *G*-statistics analogous to *F*-statistics—were also computed in combination with a 5000 times resampling strategy (with replacement), allowing to control for population and group sizes, using the “Hierstat” R package (Goudet et al. 1996; Goudet 2005) and the “sample” function (The R Project for Statistical Computing, ver. 2.3.1, <http://www.r-project.org>). A correspondence analysis considering allele identity

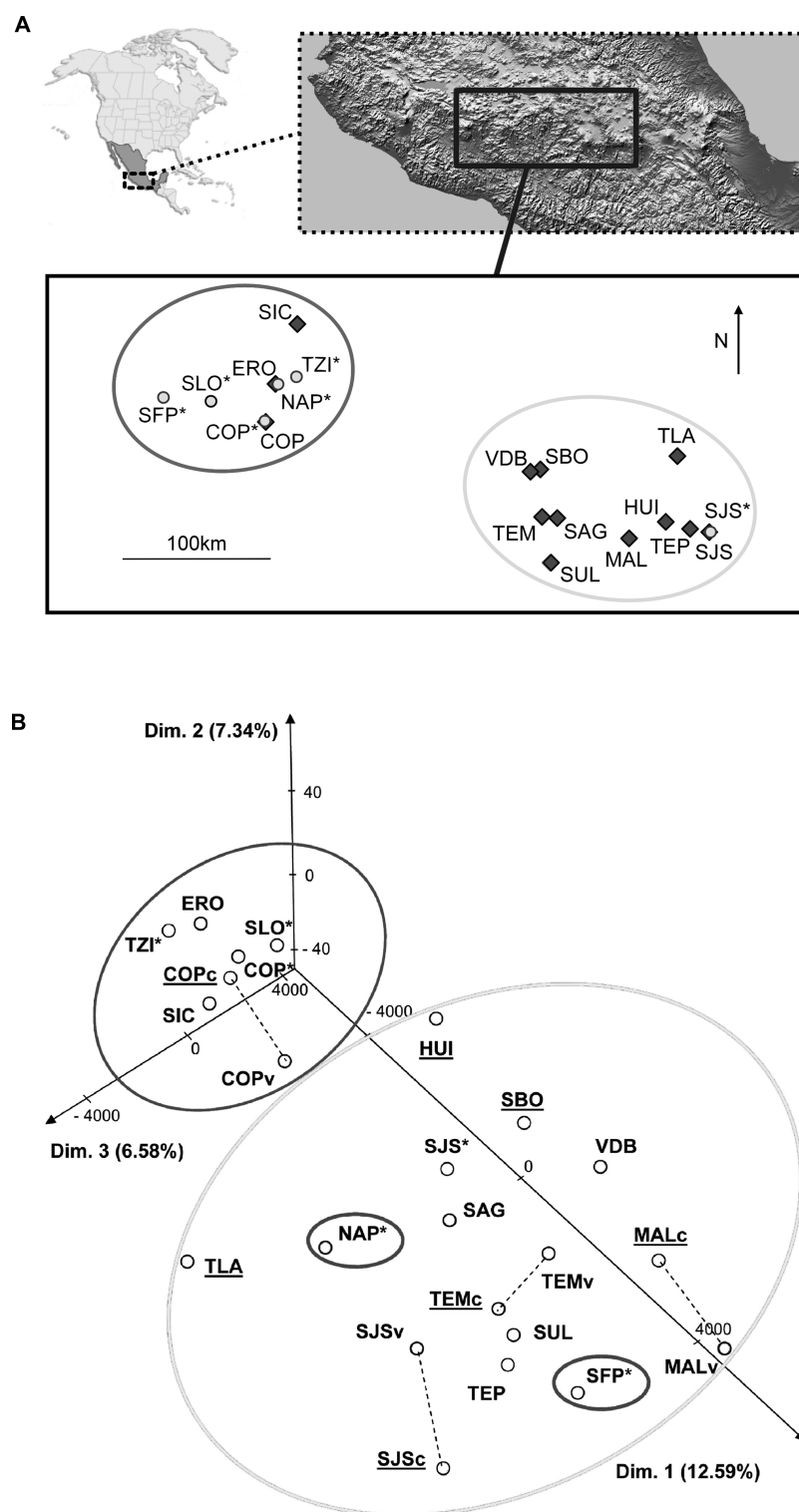


Figure 1. (A) Geographic localization of the different beetle populations sampled. Each population is referred to by a specific code (see Table 1). Dark gray diamonds correspond to populations on wild beans and light gray circles to populations on domesticated beans. Ellipses enclose populations from the northwestern part of the sampling area (dark gray ellipse) and from the southeastern part (light gray ellipse). (B) Correspondence analysis based on allelic frequencies in beetle individuals and populations. Underlined population codes refer to populations on *P. coccineus*, whereas nonunderlined population codes refer to those on *P. vulgaris*. Populations issued from domesticated beans are noted with a "*" besides the code. Subgroups collected from *P. vulgaris* and *P. coccineus* from the same site are linked by a dotted line. The dark gray ellipse surrounds populations from the northwestern part of the sampling area, whereas the light gray ellipse includes the populations from the southeastern part of the sampling area.

Table 2. Primer sequences for the three new microsatellite loci developed for *Acanthoscelides obvelatus*. Repeat motif is listed 5' to 3' with respect to the forward primer (F). "Size" refers to the length of the cloned allele.

Locus	Genbank accession number	Primer sequences (5' to 3')	Repeat motif in library	Size (bp)	Size range (bp)
AcobF11	AY730624	F: ACAGGTAAGCAGAGCATC R: GGAAATAATGAAAATACAACCTG	(TG) ₁₀	387	369–445
AcobG09	AY730623	F: GCCGTGCCGTCATATTTG R: TCTGGACGTTGGTGGTAATTTG	(AC) ₈ CTACAT(AC) ₃ GC(AC) ₃ CCGC(AC) ₂ GC(AC) ₇	351	314–366
AcobH01	AY730622	F: AGGGTTGCTGTGCGATTC R: TGCGAGCATATTCAAACAAAG	(GT) ₉ GCGG(GT) ₆	295	287–303

of each beetle individual for all loci was performed, and centers of gravity for each population were determined using Genetix 4.03 (Belkhir 2004). Geographic distances between populations were computed using the software G7twin (ver. A.00.174, R. Henderson 2002). To assess the overall IBD relationship between population pairs, a Mantel test between genetic distance (F_{ST}) and geographic distance matrices was computed using Genepop 3.4. Further Mantel tests comparing genetic distance (F_{ST}) and geographic distance were performed separately in population pairs of identical type of host plant (i.e., either both on wild host or both on domesticated host) and in pairs comprising wild and domesticated host plants. To discard any effect related to different sample size, comparison of IBD slopes for different types of host plants was carried out by resampling (with replacement) 10,000 times in the larger sample-size dataset, a number of pairs equal to the smaller sample-size dataset. We also tested at this stage the correlation between residuals from the overall IBD relationships and the kind of host-bean population. We designed an additional matrix in which pairs of beetle populations on wild beans were coded as "1" and pairs of beetle populations on domesticated beans were coded as "2" (pairs formed by a population on wild beans and a population on domesticated beans were discarded). This matrix was then compared to the matrix of residuals of the overall Mantel test previously performed, using a Kruskal–Wallis test (SAS, ver. 8.0, SAS Institute, Cary, NC). To confer less Type-I error to this analysis, the host plant matrix was resampled 100 times without replacement by randomizing the position of all "1" and "2." Kruskal–Wallis tests were then performed for each of the 100 matrices to assess whether significance could simply occur by chance.

Allelic richness (i.e., a measure of the number of alleles that is independent of sample size [El Mousadik and Petit 1996]) in populations derived from wild beans and in those from domesticated beans was calculated using FSTAT 2.9.3 (Goudet 1995). To determine whether the type of host plant was related to allelic richness, the means of allelic richness per populations of the same host type were computed. We also performed this analysis for

each locus individually, and resampled (with replacement) 5000 times in both the dataset of beetles on wild beans and the dataset of beetles on domesticated beans, a number of individuals and a number of demes equal to the smallest sample size. In addition, to test whether the total number of alleles comprised in individuals developing on one kind of host was different according to the kind of host, beetle individuals collected from a single kind of host were grouped (i.e., all individuals collected on wild beans were considered as one group and all individuals collected on domesticated beans were considered as another group). To remove sample size effect, allelic diversity in the larger group was then calculated by resampling (with replacement) 500 times, a number of individuals equal to the smallest group size (i.e., both group sizes were held constant), and further averaged. We equally performed this analysis for each locus individually, by performing a similar resampling strategy and a further Wilcoxon signed rank test. The effect of the kind of host plant on the distribution of rare alleles was also investigated by determining first which alleles were present in no more than 10% of populations. Then, the correlation between the number of rare alleles and the kind of host plant was assessed by applying an exact Wilcoxon signed rank test, without and with sample-size correction (i.e., by resampling 5000 times with replacement in the larger group a number of individuals equal to the smallest population size and a number of demes equal to the smallest group).

Bottlenecks were determined by comparing Nei's gene diversity index (a measure of heterozygosity adapted to finite populations [Nei 1987]) and allelic richness in each population, by computing Wilcoxon signed rank tests using Bottleneck 1.02 assuming an "Infinite Allele Model" with 10,000 iterations (Cornuet and Luikart 1996). When a bottleneck occurs in a population, it causes a correlative and progressive reduction of the allele number and heterozygosity. However, allelic diversity is reduced faster than is heterozygosity during a bottleneck and there is a transient deficiency in the number of alleles found in a sample of individuals (Marwama and Fuerst 1985; Luikart and Cornuet 1998).

Results

GENOTYPIC DISEQUILIBRIUM AND DEPARTURE FROM HARDY-WEINBERG EQUILIBRIUM

No genotypic disequilibrium between pairs of loci was observed in any beetle population when exact tests and a correction for multiple tests (Dunn-Sydák method for sequential Bonferroni procedure) were performed. Overall analyses for all populations and individual analyses within each population showed heterozygote deficits that departed significantly from Hardy-Weinberg equilibrium values. Overall levels of variation for each locus were as follows: 43 alleles in locus C09 (mean number of alleles/population = 15.579 ± 4.694 ; proportion of heterozygotes = 0.595); six alleles in locus D04 (mean number of alleles/population = 4.105 ± 1.049 ; proportion of heterozygotes = 0.328); 14 alleles in locus D06 (mean number of alleles/population = 8.263 ± 1.759 ; proportion of heterozygotes = 0.569); 30 alleles in locus F11 (mean number of alleles/population = 11.053 ± 3.223 ; proportion of heterozygotes = 0.402); 25 alleles in locus G09 (mean number of alleles/population = 10.737 ± 3.088 ; proportion of heterozygotes = 0.741); nine alleles in locus H01 (mean number of alleles/population = 6.316 ± 1.057 ; proportion of heterozygotes = 0.438). For the six loci, F_{IS} values in all populations ranged between 0.117 and 0.410, with no significant difference between populations on domesticated and those on wild host plants (Mann-Whitney rank sum test: $P = 0.759$). Such high values of F_{IS} , within the range considered more typical of self-fertilizing hermaphrodites than of animals with separate sexes, indicate the presence of null alleles at all loci considered, rather than breeding between close relatives. However, whereas null alleles have a significant impact on F_{IS} , they have a weak effect on F_{ST} estimates (Kelly et al. 2004). F_{ST} values among populations for all loci ranged from -0.006 to 0.121. There was no correlation between the F_{IS} value of a given population and its average pairwise F_{ST} with other populations (Spearman rank order correlation: $P = 0.4980$; $R^2 = 0.0266$).

GENETIC STRUCTURE

The correspondence analysis revealed a trend to geographic differentiation, with the populations sampled in the northwest separated from the populations sampled in the southeastern part of the study area (see Fig. 1b). F_{ST} between northwestern and southeastern populations was equal to 0.107. However, two populations on domesticated beans (NAP*, SFP*) sampled from the northwestern part of the study area were grouped together with the populations from the southeast. There was no genetic structure (i.e., no difference in allele frequencies) as a function of the kind of bean when computing standard Fisher indexes (wild vs. domesticated; $F_{ST} = 0.0015$). Although a slightly higher value of the genetic structure estimator was found when computing G -statistics and controlling for population and group sizes, differ-

entiation between populations from different kind of beans remained particularly low (wild vs. domesticated; $G_{ST} = 0.0122$; 95% confidence interval: 0.0038–0.0241; analysis performed on the basis of a resampling of 16 individuals per population and six populations per kind of bean, those values corresponding to the lowest quantities in our sampling—see Table 1). Moreover, “subgroups” collected on *P. vulgaris* and *P. coccineus* in the same site were mostly clustered together on the correspondence analysis, and F_{ST} values between these “subgroups” were noticeably low as well ($F_{ST}[\text{COPc-COPv}] = 0.0124$; $F_{ST}[\text{MALc-MALv}] = 0.0103$; $F_{ST}[\text{SJSv-SJSv}] = 0.0183$; $F_{ST}[\text{TEMc-TEMv}] = 0.0080$). Such “subgroups” were therefore pooled by site for the remaining analyses, because there was no genetic structure (i.e., no significant differences in allele frequencies) as a function of the identity of the host plant species.

ISOLATION BY DISTANCE

A Mantel test showed that geographic distances and F_{ST} values were overall significantly positively correlated ($P = 0.0005$; slope = 4×10^{-5} ; $R = 0.1226$) (overall Mantel test not shown). When considering only pairs of beetle populations on wild beans, the correlation between geographic distance and F_{ST} was stronger and showed a positive relationship with a steeper slope and larger R ($P < 10^{-4}$; slope = 0.0002; $R = 0.2453$), than when all pairs of populations were considered together (see Fig. 2). However, when only pairs of populations on domesticated beans were considered, there was no correlation between geographic distance and F_{ST} ($P = 0.2992$; slope = -5×10^{-5} ; $R = 0.0566$) (see Fig. 2). This result is in accordance with the unexpected position of two populations on domesticated beans (NAP* and SFP*) in the correspondence analysis. Finally, when only “mixed pairs” comprising wild and domesticated host plants were considered, there was no correlation between geographic distance and F_{ST} ($P = 0.5224$; slope = 1×10^{-5} ; $R = 0.0054$) (not shown). The difference between the slope obtained for pairs of populations on wild beans and that obtained for pairs on domesticated beans was not a consequence of different sample sizes (i.e., 78 and 15 population pairs on wild beans and domesticated beans, respectively), because after resampling (10,000 times) 15 pairs among pairs on wild beans, the average slope for population pairs on wild beans was significantly steeper ($P = 0.0021$; i.e., the IBD slope in the larger sample-size [wild] group was higher than that of the smaller sample-size [domesticated] group in 9979 of the 10,000 resamplings).

When considering the overall Mantel test (consisting in the global correlation between F_{ST} and geographic distance when taking all population pairs into account; slope = 4×10^{-5} and $R = 0.1226$ [not shown]), the residuals—in which the amount of variance explained by geographic distance is removed—were also significantly different between pairs of populations on wild beans

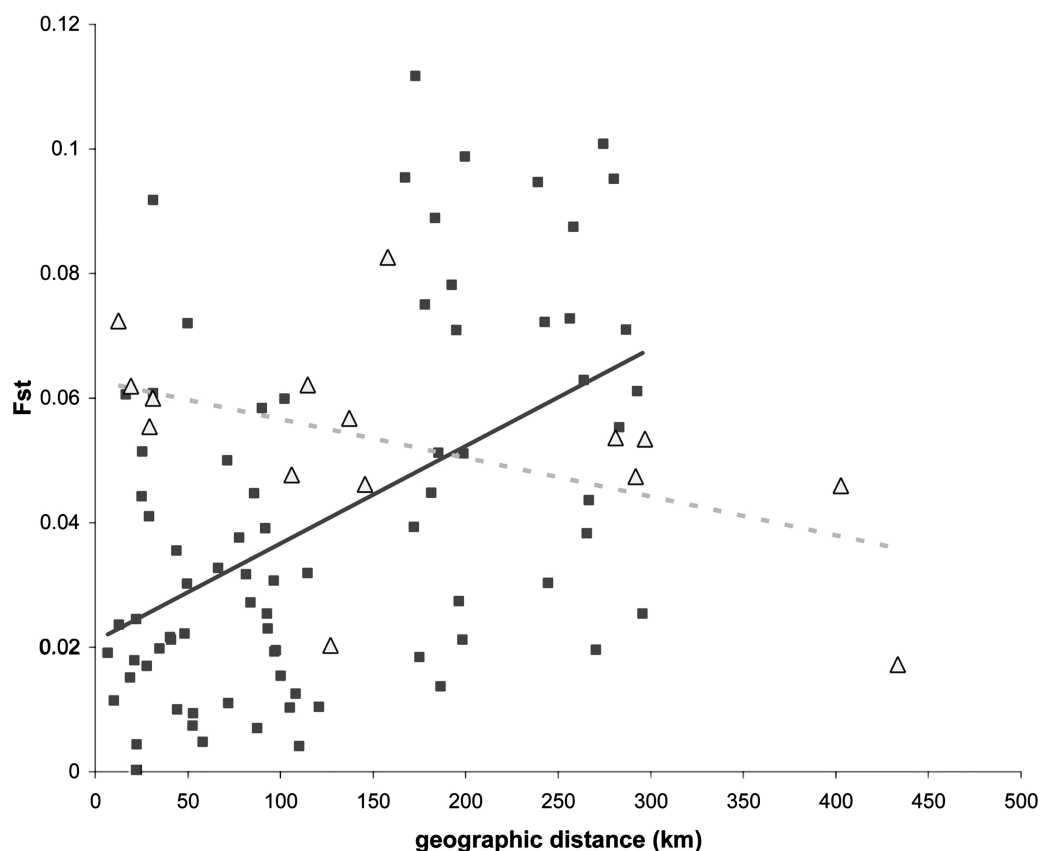


Figure 2. Genetic differentiation (F_{ST}) between pairs of populations according to geographic distance (km). Dark gray squares refer to pairs in which the two beetle populations develop on wild beans, and light gray triangles correspond to pairs in which the two beetle populations develop on domesticated beans. The dark gray line is the regression slope for pairs of populations feeding on wild beans. The light gray dotted line is the regression slope (nonsignificant) for pairs of populations feeding on domesticated beans.

and pairs of populations on domesticated beans, being greater for pairs of populations on domesticated beans (Kruskal–Wallis test: $\chi^2_{1df} = 4.3259$; $P = 0.0375$). This correlation was not artifactual because only one among the 100 resampled host plant matrices yielded a significant P value (see Appendix 1), this value being moreover associated with a lower chi-squared value than in the test based on the original host plant matrix.

ALLELIC RICHNESS AND DIVERSITY

Mean allelic richness in beetle populations derived from domesticated beans (6.93 alleles per locus and per population) and in populations from wild beans (7.46 alleles per locus and per population) was not significantly different on average ($P = 0.304$). When considering each locus individually and controlling for population and group sizes, no differences in allelic richnesses were found as well (see Appendix 2). However, the overall number of alleles in beetles from wild beans (20.09 alleles per locus)—computed after resampling 500 times, 135 individuals (i.e., the sample size of the smallest group [composed of populations on domesticated beans]) in the group of populations on wild beans (i.e., the larger group with 359 individuals)—was significantly higher

($P = 0.002$) than that in the entire sample from domesticated beans (18.40 alleles per locus). Similarly, when looking at each locus individually and controlling both for population and group sizes (i.e., by resampling 16 individuals per population and six populations per group), a higher overall number of alleles was found in beetles on wild beans, at four of the six loci analyzed (i.e., highly significant in C09, D04, F11, and G09 [Wilcoxon signed rank test: $P < 10^{-4}$], not significant in D06 and H01). Furthermore, the mean number of rare alleles (defined as those present in no more than $\sim 10\%$ of all populations for each locus—that is, alleles present in one [5.26%] or two [10.52%] populations among the 19 studied sites) was significantly greater in beetle populations from wild beans than in those from domesticated beans (number of rare alleles per locus in beetles on wild beans \pm standard deviation = 4.8 ± 2.8 ; number of rare alleles per locus in beetles on domesticated beans = 1.6 ± 1.5 ; Wilcoxon signed rank test; $P = 0.0384$). This result was even magnified when correcting both for the number of individuals per population and for the number of populations in each group (Wilcoxon signed rank test: $P < 10^{-4}$).

After Bonferroni correction, two of the six beetle populations on domesticated beans exhibited significant bottlenecks (SFP*

[uncorrected $P = 0.0078$; Bonferroni corrected $P < 0.05$] and SJS* [uncorrected $P = 0.0078$; Bonferroni corrected $P < 0.05$]. In contrast, no bottleneck was detected for any of the 13 beetle populations on wild beans after Bonferroni correction. The differences in bottleneck patterns between populations on wild versus domesticated bean (i.e., allelic richness and diversity) are the consequence of a faster reduction of allelic diversity than of heterozygosity in one-third of populations on domesticated beans.

Discussion

Our results demonstrate unambiguously a difference in IBD depending on the wild or domesticated status of the host plants of beetle populations, and an interesting complementary result on the nature of the allelic diversity between beetles on wild beans and on domesticated beans.

The overall positive relationship found between genetic differentiation and geographic distance is consistent with Wright's IBD theory. However, this correlation is only due to the IBD occurring between population pairs on wild beans. There is not even a trend toward such a correlation between genetic differentiation and geographic distance when only pairs of populations on domesticated beans are considered; the slope of this regression is negative. This difference between population pairs on wild beans and population pairs on domesticated beans is corroborated by the relationship arising between the residuals of the overall Mantel test and the kind of host plant: on average, points situated close to the overall regression line (not shown) correspond to pairs of populations on wild beans, whereas points situated far from the line correspond to pairs of populations on domesticated beans. The absence of IBD in populations originating from domesticated beans is most likely explained by frequent long-distance human-mediated migrations of bean seeds (long-distance human exchanges also modify population structure of the bean itself [see Gepts 1998; Papa and Gepts 2003]). Indeed, *A. obvelatus* individuals can emerge very far geographically from the bean field in which they originated, if farmers exchange seeds or sell seeds in local or regional markets. Such seed exchanges can be a driving force of migration for beans and associated beetles, which can thereby travel hundreds of times farther than by natural means. Interestingly, migration also occurs homogeneously between beetle populations on wild and domesticated beans, because no IBD was detected in these "mixed pairs" either (although a trend was observed). Beetles migrating through human trade may indeed reach wild beans by natural dispersal after emergence, assuming that wild beans are spatially close to human-inhabited areas. Within the range of our study (500 km), individuals emerging from domesticated beans can potentially interbreed with any conspecific developing on other domesticated beans—and occasionally with conspecifics developing on wild beans—almost inde-

pendently of the geographic distance. Such long-range gene flow through extensive migration appears to have homogenized the intrapopulation genetic diversity of beetle populations on domesticated beans. As a result of this homogenization, allelic richness in populations (i.e., within a single deme) on domesticated beans is not different from that in populations on wild beans, despite the strong drift that must occur in seed granaries. Such drift in populations on domesticated beans appears to result from strong demographic stochasticity, as attested by the bottlenecks detected in one-third of the analyzed populations on domesticated beans (but in none of the beetle populations on wild beans). It should be noted that bottlenecks are detected in populations that have experienced a recent reduction of their effective population size, because allelic diversity is reduced faster than is heterozygosity (see Cornuet and Luikart [1996]). As a consequence, in such populations, the heterozygosity computed from a sample of genes is larger than the heterozygosity expected from the number of alleles found in the sample. However, assuming moderate levels of immigration—as shown in beetle populations on domesticated beans (see above)—allelic diversity should be recovered relatively rapidly (Keller et al. 2001). In such conditions of gene flow (although the role of migration on the power within which a bottleneck can be detected is still debated [e.g., Ramakrishnan et al. 2005]), a difference between allelic diversity and heterozygosity is even more difficult to identify, giving a stronger weight to the identification of bottlenecks. Bottlenecks may be due to the use of insecticides, human seed consumption, and predation during migrations from granaries to bean fields or to wild bean populations. Without the extensive migration resulting from seed exchanges, this drift should have led to among-population differentiation and loss of genetic diversity. In contrast, individuals emerging from wild beans can disperse only by natural means and at short distances, such low migration rate leading to differentiation among populations.

Similarity in allelic richness of populations on wild beans and those on domesticated beans thus appears to be the consequence of different population processes. A remarkable result is that the nature of allelic diversity is however not identical between populations of beetles on wild beans and those on domesticated beans. Indeed, the overall weighted allelic diversity among individuals from all beetle populations on wild beans was significantly higher than among individuals from domesticated beans. This outcome is confirmed by the fact that the number of rare alleles (i.e., those present in no more than ~10% of all populations) was significantly greater in populations originating from wild beans (i.e., more likely to demonstrate relatively constant sizes) than in populations originating from domesticated beans.

This study reveals how human practices have contributed to shape the genetic structure of populations of phytophagous insects associated with domesticated plants. Large differences in population processes have resulted from contrasting patterns of drift

and migration in populations on wild and domesticated beans. To summarize, whereas on wild beans, constant beetle population sizes result in low effect of drift, and migration rates are low (i.e., differentiating migration), in contrast, on domesticated beans, bottlenecks are frequent (which means that drift has a strong effect) and migration rates are high (i.e., homogenizing migration). As a result, allelic diversity at the level of a single deme is similar between the two kinds of populations, but structured differently. Population processes in demes on wild beans allow the persistence of rare alleles and a high overall allelic diversity, whereas strongly contrasting processes in demes on domesticated beans only maintain frequent alleles (i.e., most populations on domesticated beans share the same alleles), and a lower overall allelic diversity.

The absence of IBD between beetle populations originating from domesticated beans combined with a moderate average F_{ST} value of ~ 0.05 among populations on domesticated beans (see Fig. 2) suggests that beetle individuals emerging from domesticated beans can be considered as belonging to a single gene pool. Therefore, selection would be expected to act on a much greater effective population size, and an adaptive mutation that emerges would be quickly spread in all beetle individuals on domesticated beans. If such a mutation were counter-selected on wild beans, its spread could be followed by the formation of host races (with some individuals being particularly adapted to domesticated beans and the others remaining on wild beans; e.g., such differences in beans are attested chemically and morphologically [Calderon et al. 1992; see also Papa and Gepts 2003]) or even to speciation. Strong selection on what is effectively a single large population of beetles on domesticated beans could also enable rapid dissemination of resistance genes—for example to GM host plants or insecticides—acquired by beetles. Hence, if an adaptive resistance gene were to appear in any beetle population on domesticated beans, such a gene would rapidly spread, rendering the insecticide or the GM plant nonuseful.

Seed exchange is already recognized as an important factor molding genetic structure of domesticated plants (e.g., Louette et al. 1997; Alvarez et al. 2005b). In this study we present evidence that such an exchange also affects the structure of populations of a phytophagous insect associated with a domesticated plant. Similar patterns are likely to be found in other organisms associated with both domesticated plants and their wild relatives, such as other phytophagous insects and their natural enemies, microbial pathogens and microbial symbionts.

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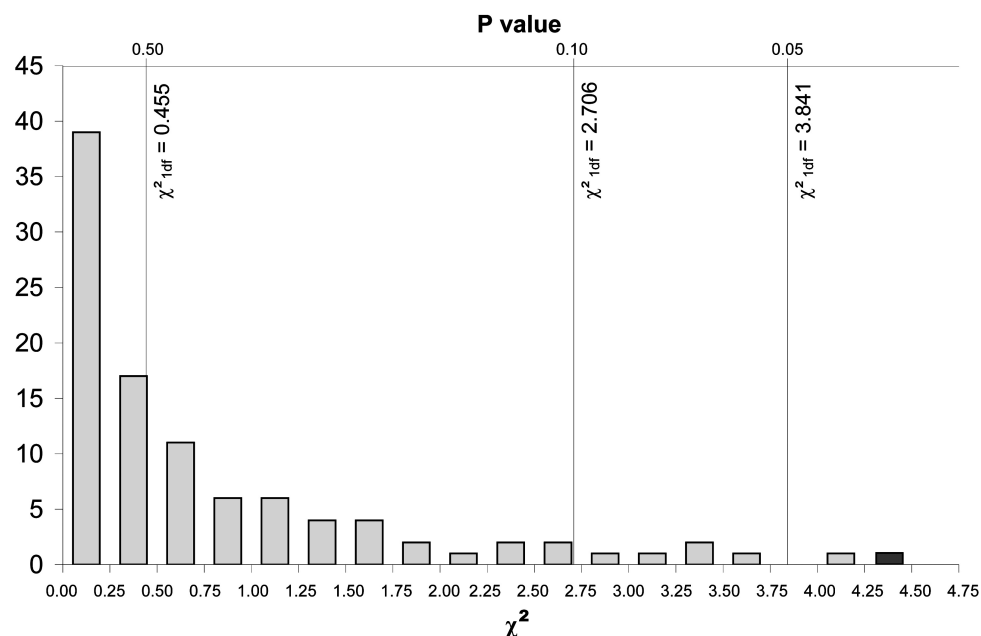
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Appendix 1. Histogram of the chi-square values corresponding to the Kruskal–Wallis test performed to assess the correlation between Mantel test residuals of the IBD and the kind of host plant. Light gray bars correspond to tests performed for 100 different shuffled host plants matrices, whereas the single dark gray bar corresponds to the real data. Vertical lines address chi-square values associated with $P = 0.5$, $P = 0.1$, and $P = 0.05$.

Appendix 2. Allelic richness at each locus, in populations on wild beans and in populations on domesticated beans. Means \pm standard deviation and 95% confidence intervals were computed after performing 5000 times resampling of 16 individuals per population and six populations per kind of beans (i.e., those values corresponding to the smallest quantities in our sampling).

Locus	Allelic richness in beetles on wild beans \pm SD [95% confidence interval]	Allelic richness in beetles on domesticated beans \pm SD [95% confidence interval]
C09	24.65 \pm 2.7 [19–30]	24.44 \pm 1.8 [21–28]
D04	5.52 \pm 0.6 [4–6]	5.35 \pm 0.6 [4–6]
D06	10.80 \pm 1.2 [8–13]	11.70 \pm 0.9 [10–13]
F11	17.17 \pm 2.3 [13–21]	14.94 \pm 1.2 [13–17]
G09	15.71 \pm 1.9 [12–19]	14.13 \pm 1.4 [12–17]
H01	7.24 \pm 0.6 [6–8]	8.01 \pm 0.7 [7–9]