*Eur. J. Entomol.* **107**: 299–304, 2010
http://www.eje.cz/scripts/viewabstract.php?abstract=1540
ISSN 1210-5759 (print), 1802-8829 (online)

# The effect of host plant and isolation on the genetic structure of phytophagous insects: A preliminary study on a bruchid beetle

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**Key words.** Phytophagous insects, Bruchidae, *Acanthoscelides*, spatial genetic structure, gene flow, adaptation

**Abstract.** Genetic differentiation is a consequence of the combination of drift and restriction in gene flow between populations due to barriers to dispersal, or selection against individuals resulting from inter-population matings. In phytophagous insects, local adaptation to different kinds of host plants can sometimes lead to reproductive isolation and thus to genetic structuring, or even to speciation. *Acanthoscelides obtectus* Say is a bean bruchid specialized on beans of the *Phaseolus vulgaris* group, attacking both wild and domesticated forms of *P. vulgaris* and *P. coccineus*. This study reveals that the genetic structure of populations of this bruchid is explained mainly by their geographical location and is not related to a particular kind (wild or domesticated) of bean. In contrast, the species of bean might have led, to some extent, to genetic structuring in these bruchids, although our sampling is too limited to address such process unambiguously. If confirmed, it would corroborate preliminary results found for the parasitoid species that attack *Acanthoscelides* species, which might show a genetic structure depending on the species of host plant.

## INTRODUCTION

Spatial genetic structure is a consequence of drift and reproductive isolation that occurs when gene flow between populations is restricted, due to geographic barriers limiting dispersal, or to counter-selection of individuals resulting from inter-population matings induced by differential selection among populations. In the last decade, numerous studies have examined restricted gene flow in phytophagous insects in natural populations on wild plants as well as in pest species attacking wild (e.g., Peterson & Denno, 1998; Keyghobadi et al., 1999; Peterson et al., 2001; Brouat et al., 2003) or domesticated plants, (e.g., Sembene & Delobel, 1998; Kerdelhué et al., 2002; Cognato et al., 2003; Nahrung & Allen, 2003). However, few studies have investigated the genetic differentiation in phytophagous insects attacking both domesticated and wild populations of host-plants (Gonzalez-Rodriguez et al., 2000; Alvarez et al., 2007). Plant domestication has led to important changes, in many plant traits, including morphology as well as the nature and quantity of both nutrients and defensive chemical compounds [e.g., Smartt (1988) in beans, Benrey et al. (1998) in *Brassica* and *Phaseolus*]. Divergence of domesticated populations from their wild parents could drive local adaptation in populations of associated phytophagous insects, with some populations being adapted to these new characteristics of domesticated host plants (Mopper, 1996).

Bruchid beetles feeding on beans are an appropriate model to test local adaptation to wild and domesticated host plants since they are usually oligophagous or monophagous, with a very tight relation with the seeds of their hosts. They should therefore be very sensitive to modifications in the secondary compounds and morphology of seeds, modifications that are common in the grain of domesticated legumes (i.e., domestication syndrome) (Smartt, 1988). The genus *Acanthoscelides* is one of the largest bruchid genera comprising about 300 species, all of which originated from neotropical regions (Alvarez et al., 2006a). Among them, the multivoltine and worldwide distributed *A. obtectus* Say is a specialist on beans of the *Phaseolus vulgaris* L. group (Pichard et al., 1991; Alvarez et al., 2005). In the Neotropical region, where this species originates, larvae develop on both wild and domesticated forms of the two *Phaseolus* species most frequently encountered, *P. vulgaris* and *P. coccineus*. These two bean species occur at different altitudes, *P. vulgaris* mostly at low altitudes and *P. coccineus* at high altitudes (Delgado-Salinas et al., 1988). Comparative studies of the chemical compounds in beans have revealed numerous quantitative differences between *P. vulgaris* and *P. coccineus* (especially regarding cyanogenic compounds and toxic proteins; Calderon et al.,

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TABLE 1. List of sites sampled for *A. obtectus*. The kind of bean is referred to as "D" for domesticated and "W" for wild.

| Code | Site name | Sample size | Bean species | Kind of bean | Longitude | Latitude | Altitude (m) |
|---|---|---|---|---|---|---|---|
| COE | Coeneo, MEXICO | 23 | *P. vulgaris* | D | 101°34′59.3″W | 19°49′13.9″N | 2100 |
| MALc | Malinalco, MEXICO | 14 | *P. coccineus* | W | 99°30′08.9″W | 18°57′13.2″N | 1935 |
| MALv | Malinalco, MEXICO | 12 | *P. vulgaris* | W | 99°30′08.9″W | 18°57′13.2″N | 1935 |
| SAG | San Andres de los Gabeles, MEXICO | 11 | *P. vulgaris* | W | 99°57′01.5″W | 19°02′19.5″N | 2280 |
| SIC | San Isidro cerca Coeneo, MEXICO | 18 | *P. vulgaris* | W | 101°34′23.9″W | 19°50′56.6″N | 2040 |
| SPT | San Pablo de Tejalpa, MEXICO | 22 | *P. vulgaris* | D | 99°36′00.3″W | 18°52′59.8″N | 1750 |
| STM | Santa Maria, MEXICO | 24 | *P. vulgaris* | D | 99°33′42.7″W | 18°49′03.6″N | 2000 |
| TEJ | Tejupilco, MEXICO | 18 | *P. vulgaris* | D | 100°09′00.1″W | 18°55′51.2″N | 1400 |
| TEP | Tepoztlan, MEXICO | 24 | *P. vulgaris* | W | 99°07′15.7″W | 18°59′36.3″N | 1931 |
| TLA | Tlalpan, MEXICO | 10 | *P. coccineus* | W | 99°12′04.3″W | 19°17′50.3″N | 2403 |
| XOT | Xochitlan, MEXICO | 23 | *P. vulgaris* | D | 97°39′02.0″W | 19°57′59.9″N | 1450 |
| MAM | Mamorimamba, PERU | 180 | *P. vulgaris* | D | 78°48′33.6″W | 6°30′42.2″S | 1900 |
| BEL | Bellevue, FRANCE | 23 | *P. vulgaris* | D | Région du Poitou, FRANCE | | |
| CHA | Chail, FRANCE | 21 | *P. vulgaris* | D | Région du Poitou, FRANCE | | |
| NEU | Chambrelien, SWITZERLAND | 15 | *P. vulgaris* | D | Canton de Neuchâtel, SWITZERLAND | | |
| PRA | Prado, SPAIN | 21 | *P. vulgaris* | D | Provincia de León, SPAIN | | |

1992), and between wild and domesticated kinds of beans (Sotelo et al., 1995). In the present study, it is hypothesized that differences in chemical compounds between populations of host plants, either as a function of the species (*P. vulgaris* vs. *P. coccineus*) or of the kind (wild vs. domesticated) of beans, could exert a sufficiently strong selection pressure resulting in genetic structuring in *Acanthoscelides* populations, as shown experimentally in other bruchids species (e.g. *Zabrotes subfasciatus*; Benrey et al., 1998 ).

Microsatellite markers were used to examine whether the identity of host plant species or the kind of bean is related to genetic structuring among populations of *A. obtectus.* Gonzalez-Rodriguez et al. (2000) examined the genetic structure of populations of *A. obtectus* using allozyme markers and found no structuring as a function of the host plant. The question of host race formation is re-examined here using more variable microsatellite markers, whose high mutation rate could provide more information on the evolution of recent local adaptation. Indeed, if selection pressures differ among groups (i.e. bean kind or species) then the realized gene flow among the different compartments due to counter-selection could be low. In this study a wide range of habitats, including other parts of South America and Europe, were sampled. The aim is to evaluate the extent to which the genetic structuring of *A. obtectus* is explained by geography and/or by the host plant and to precise the scale at which these effects occur.

## MATERIAL AND METHODS

### Sampling of *A. obtectus*

Four hundred and sixty *A. obtectus* individuals were collected from 16 populations in Mexico, Peru, and Europe (see Table 1) between December 2001 and March 2003. Although only *P. vulgaris* is grown in most places in Europe, several populations were included to compare host-plant structuring and geographic structuring. All Peruvian individuals [originating from one population (MAM*)] and all European individuals [originating from four populations (PRA*, BEL*, CHA*, NEU*)] fed on seeds of domesticated *P. vulgaris*, whereas in Mexico, five populations fed on domesticated *P. vulgaris* (COE*, SPT*, STM*, TEJ*, XOT*), three on wild *P. vulgaris* (SAG, SIC, TEP), one on wild *P. coccineus* (TLA), and one on mixed bean populations of both *P. vulgaris* and *P. coccineus* (MAL). In a first approach we considered that the mixed bean population harbored two distinct populations of *A. obtectus*. Since *P. coccineus* grows at relatively high altitudes only two sites were found where there were only wild plants, which where fed on by *A. obtectus*.

### Genetic analysis

DNA of insects was extracted using a DNEasy Tissue Kit (Qiagen, Bassel, Switzerland). Five microsatellite loci (*AcobtC12*, *AcobtE01*, *AcobtE07*, *AcobtF09*, and *AcobtG08*) were amplified according to Alvarez et al. (2004). Genotyping was done using an ABI PRISM 310 sequence analyzer (Applied Biosystems, Foster City, CA, USA). Results were analyzed with Genescan 3.1.2 and Genotyper 2.5 (Applied Biosystems) softwares, and exact values of allele sizes were rounded to closest integers after plotting exact allele sizes and determining the size mode of each allele.

Genotypic disequilibrium between loci and deviation from Hardy-Weinberg equilibrium were tested using Genepop 3.4 (Raymond & Rousset, 1995). A factorial analysis of correspondence considering the frequency of each allele within populations was conducted using Genetix 4.05 (Belkhir, 2004). To infer genetic structuring of populations AMOVAs (Excoffier et al., 1992) were computed using Arlequin 3.11 (Excoffier et al., 2005) in order to test for the significance of groups of populations defined on the basis of biological assumptions. The three following hypotheses were tested: 1. genetic structure is due to the host-plant type, grouping populations according to the kind of bean (wild type *vs.* domesticated type); 2. genetic structure is a consequence of the host-plant species, categorized by the host species, *Phaseolus vulgaris* or *P. coccineus*, among wild types of bean populations (since the latter species was only found as a wild species); 3. bruchids are structured according to the geographical pattern at a large scale (here, the three following areas were defined, Peru, Mexico, and Old World); hypotheses 1 and 2 were tested using Mexican populations, in order to avoid con-

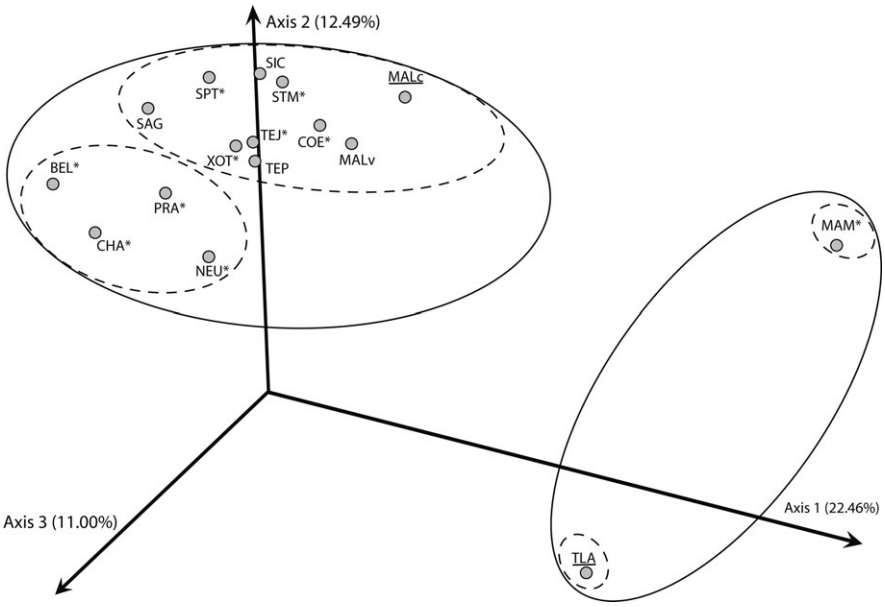


Fig. 1. Correspondence analysis based on allelic frequencies in populations of *A. obtectus*. Each population is referred to by a specific code (see Table 1). Underlined population codes refer to populations on *P. coccineus* and non-underlined codes to those on *P. vulgaris*. Populations that originated from domesticated beans have a "*" beside the code. The external circles (complete lines) enclose groups of populations that segregated with $K = 2$ groups in Structure 2.2 (Falush et al., 2007). The internal circles (dashed lines) enclose groups of populations that segregated with $K = 4$ groups. Group assignment followed the Majority-Rule criterion. i.e., a population was assigned to a group when its assignment probability was higher than 0.5 (exact probability values are available from the first author on request).

founding effects of geography and host-plant. For each hypothesis, the percentage of variance explained by groups was computed. To account for unbalanced sample sizes, significance of this latter index was tested using the permutation algorithm implemented in Arlequin (Excoffier et al., 2005).

Finally, the genetic structure of all sampled individual was tested using a model-based Bayesian approach in STRUCTURE 2.2 (Falush et al., 2007). The aim of this software is to assign individuals to one or more groups, by optimizing the Hardy-Weinberg equilibrium within groups. The number of groups, *K* (that requires to be previously defined by the user) was set from one to sixteen (i.e. the total number of sampled populations). MCMC simulations were performed using the admixture model (i.e. individuals can be assigned to more than one group) and correlated genetic frequencies among individuals. Simulations ran for 1,000,000 iterations using 100,000 iterations as a burn in period (that was not taken into account in the analysis). The majority-rule criterion (> 0.5 in the assignment probability) was applied to assign a population to a given group.

## RESULTS

Four of the five microsatellite loci (*AcobtC12*, *AcobtE07*, *AcobtF09*, and *AcobtG08*) were correctly amplified for all 460 individuals and were polymorphic. However, locus *AcobtE01* did not amplify consistently in all populations and was not included in the analysis. After Bonferroni correction, there was no significant genotypic disequilibrium between any pair of loci. The spatial representation of the three first axes of the population-based factorial correspondence analysis (about 46% of the total variance explained) clustered European and Mexican (except individuals from TLA) populations together, whereas the Peruvian population and that collected in a public park located in the center of Mexico City (TLA), were isolated from all other populations, particularly on the first axis (see Fig. 1). Although Mexican populations were mostly grouped together, they were more dispersed than European populations probably because the number of sampled populations of the Mexican group was greater than that of the European group. However, European and Mexican groups were well segregated in this analysis (mainly based on the third axis). Nonetheless, the TLA Mexican population was well separated from the others and seemed to be closer to the Peruvian populations than

TABLE 2. Results of the AMOVA testing for geographic structure in *A. obtectus* populations. Localities were categorized in one of three regional areas, Peru, Mexico, and Old World. For each level the number of degrees of freedom, the sum of squares, the variance components (i.e. the variance explained by a given level), and the proportion of variance explained by the level in the global model, are presented. Values in the table are averaged to two decimal places.

| Source of variation | Degrees of freedom | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among localities | 2 | 46.03 | 0.13 | 8.20 |
| Among populations – within localities | 13 | 61.91 | 0.18 | 11.60 |
| Within populations | 346 | 438.61 | 1.27 | 80.19 |
| Total | 361 | 546.55 | 1.58 | |

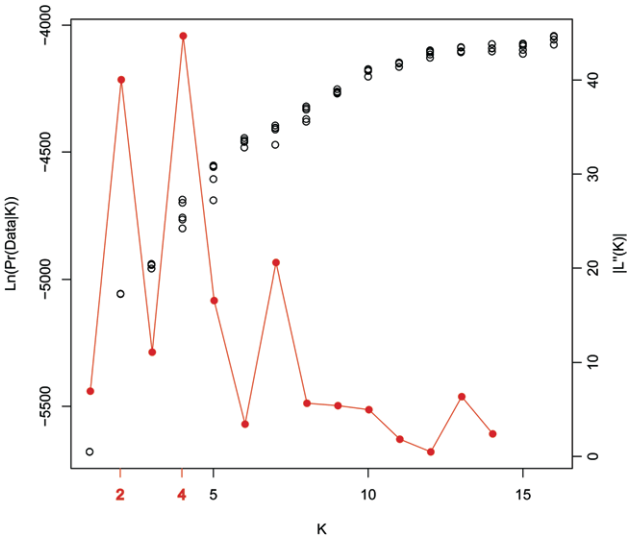


Fig. 2. Likelihood values for each number of groups tested (i.e. the *K* value) with Structure 2.2. The empty circles represent the exact likelihood values for each run with *K* ranging from 1 to 16. Filled circles and complete line represent the absolute value of the approximate of the second derivative of the mean likelihood for each tested *K* value according to Evanno et al. (2005): maximum values (i.e. most optimal number of groups) correspond to $K = 2$ and $K = 4$.

to the others. Populations on wild beans and those on domesticated beans were not differentiated by the correspondence analysis.

These trends were confirmed by the AMOVA results: populations were primarily grouped according to their geographic locations. Indeed, 8.20% ($P = 0.004 \pm 0.002$) of the total variance component was explained by the geographical regions (i.e. Peru, Mexico, and Old World) (see Table 2). When looking at a possible structure by host-plant types (i.e. domesticated beans vs. wild beans) or by host-plant species (i.e. *P. vulgaris* vs. *P. coccineus*) within the Mexican populations, the differences among groups were not significant, with respectively 0.27% ($P = 0.44 \pm 0.005$, Table 3) and 17.67% ($P = 0.068 \pm 0.003$) of the total variance explained. Although this latter effect was marginally non significant, contrasting patterns were observed when analyzing population genetics parameters at different spatial scales: at the scale of Mexico, the TLA population differed from the others in that bruchids presented a low and non significant $F_{IS}$ ($F_{IS} = 0.09$, $P = 0.43$) and a marginally non significant *Fst* when compared to all other Mexican specimens on wild beans pooled together ($F_{ST} = 0.225$ ; $P = 0.092 \pm 0.003$). When focusing on the MAL population, a significant genetic differentiation was observed between individuals feeding on *P. vulgaris* and on *P. coccineus* ($F_{ST} = 0.24$ ; $P = 0.03 \pm 0.002$).

Finally, the individual-based Bayesian-inferred genetic structure showed a similar pattern with a marked geographical segregation of populations. When applying Evanno's method of estimating the optimal number of groups (Evanno et al., 2005), two ways of structuring the populations were found, either into two groups (i.e. $K = 2$) or four groups (i.e. $K = 4$) (see Figs 1 and 2). In the first (i.e., $K = 2$), the Peruvian population and the TLA population from Mexico City were isolated from the others. However, in the second (i.e., $K = 4$), the four clusters were defined as follows: European individuals, Mexican individuals (with the exception of those proceeding from TLA), Peruvian individuals, and individuals from the Mexican TLA population.

## DISCUSSION

### Effect of the kind of host-plant

At the Mexican scale, the results unambiguously indicate that the kind of host-plant has no effect on the genetic structure of populations. Thus domestication of beans does not seem to have affected the genetic structure of associated bruchids. This result contrasts with the nutritive and chemical differences between domesticated and wild beans (Sotelo et al., 1995), which might have led to specific adaptations in phytophagous species or pathogens (Benrey et al., 1998; Lindig-Cisneros et al., 2002). Indeed, domesticated beans and their wild relatives are morphologically and physiologically distinct (Smartt, 1988; Benrey et al., 1998), and as there is little gene flow between these two groups (Gepts et al., 1999) they have different gene pools. Using another bruchid beetle developing on *Phaseolus* beans, Alvarez et al. (2007) show that the genetic diversity and structuring of this insect is driven by different processes in the populations feeding on wild (limited dispersal leading to isolation by distance, IBD) or cultivated beans (strong drift and human-mediated migration over long-distances without IBD). In contrast, in the present study, spatial proximity between wild and domesticated bean fields led to high levels of gene flow and thus to comparable levels of genetic diversity and no genetic differentiation even if a stronger drift in the domesticated populations, due to farming practices, could lead to the loss of rare alleles.

TABLE 3. Results of the AMOVA testing for the effect of the kind of host-plant on the genetic structure of populations of *A. obtectus*. The kind of host-plant is defined as wild or domesticated. For each level the number of degrees of freedom, the sum of squares, the variance components (i.e. the variance explained by a given level), and the percentage of variance explained by the level in the global model, are presented.

| Source of variation | Degrees of freedom | Sum of squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among kinds of host-plant | 1 | 3.147 | 0.00385 | 0.27 |
| Among populations within host-plant kind | 7 | 19.998 | 0.05477 | 3.81 |
| Within populations | 77 | 141.338 | 0.45848 | 31.93 |
| Within individuals | 86 | 79 | 0.9186 | 63.98 |
| Total | 171 | 243.483 | 1.4357 | |

### Effect of the host-plant species

The effect of the bean species on the genetic structure of associated bruchids is more difficult to study than the effect of the kind of beans, since the dataset was not initially designed for this purpose. Overall there is a marginally non significant genetic differentiation between populations of *A. obtectus* feeding on *P. vulgaris* and *P. coccineus*. The latter populations included the TLA population that is located in the center of Mexico City and is genetically different from all other populations. This pattern indicates a possible effect of migration and drift in the population from Mexico City (TLA), as it is likely that it originated from beans cultivated all over the continent and dispersed through human-mediated trade. This could explain the remarkably low and non significant fixation index ($F_{IS}$), as well as a possible bottleneck attested by the monomorphic nature of alleles in one among four microsatellite loci (data not shown) in this population. Urban fragmentation might have isolated the TLA population more than any other and drift and migration could have been important in the loss of rare alleles and conservation of a high level of genetic diversity (Alvarez et al., 2007). Thus to remove such confounding effects potentially induced by the comparison of populations differing in terms of host-plant species and localities (Delgado-Salinas et al., 1988; Alvarez et al., 2006b), individuals sampled at the same locality (MAL), for which bruchids emerged from both *P. vulgaris* and *P. coccineus,* were compared. In this case, a significant genetic structure according to the host-species was found. However, the gene flow between the lineages on *P. vulgaris* and on *P. coccineus* must be important because of their spatial proximity. Thus the genetic differentiation we detected could be induced by host preferences at the species level. Nonetheless, this result contrasts with Alvarez et al. (2007) who did not find any differentiation between populations of *A. obvelatus* feeding on *P. vulgaris* or *P. coccineus* at the same locality. Differences in life history traits (notably the level of voltinism, with *A. obtectus* polyvoltine and *A. obvelatus* univoltine) might explain the contrasting patterns found in these two species. Thus genetic differentiation between populations feeding on *P. vulgaris* and *P. coccineus* could occur more rapidly in *A. obtectus* than in *A. obvelatus*, due to the higher rate of generation turnover. Interestingly, Aebi (2004) found a differentiation between bean bruchids parasitoids according to the host-plant species on which they were collected, which indicates a potential influence of host-plant species.

### Spatial genetic structure of populations of *Acanthoscelides obtectus* at a large scale

As expected *Acanthoscelides obtectus* populations are genetically structured at the scale of the whole distribution area. Indeed without assuming an *a priori* spatial genetic structure (SGS hereafter), four STRUCTURE clusters were obtained: most of the Mexican populations (except TLA population) were clearly clustered and separated from European populations, which also formed a solid cluster. These two clusters were genetically isolated from the Peruvian population (MAM). Finally, the analysis revealed a fourth group composed only of individuals originating from the TLA population, collected in the center of Mexico City. However, due to the location of this population in an urban area where most beans are imported from elsewhere, even far away places, the specimens might have had a strong admixture of highly diverging gene pools. The analysis supported an SGS based on three clusters defined as Peruvian, European, and Mexican. The geographical isolation of these groups of populations could have led to genetic divergences due to founder effects (in particular, for European populations where *A. obtectus* was introduced together with the beans in the XVI$^{th}$ century; Smartt & Simmonds, 1995). It is also likely that selection might be different due to the very different climates, leading to larger divergence among groups.

## CONCLUSIONS AND PERSPECTIVES

This study indicates that the genetic diversity of populations of *A. obtectus* is mainly geographically structured. In addition, the results highlight the importance of founder effects, barriers to gene flow, and human mediated gene flow leading to spatial differences in genetic structure at a very large scale. Host-plant species also seem to slightly influence the genetic structure of associated bean bruchids, whereas the kind of bean does not. As few *A. obtectus* populations feeding on *P. coccineus* were investigated, these conclusions must be strengthened in the future by studying larger samples of the populations. However, this work indicates that voltinism is possibly an important structuring life history trait, and its precise role in determining the fine spatial genetic structure of the *Acanthoscelides* genus and of pests in general merits further study. Multivoltinism could enhance the capacity of a species to quickly adapt to a new environment and in the case of pest species the spread of alleles providing resistance to pesticides. In the future, the relationship between host-plant phenology and level of voltinism of associated pest insects should be more thoroughly investigated experimentally. A comparative study including other Mesoamerican *Acanthoscelides* species (e.g., *A. argillaceus* and *A. obvelatus*, also specialized on *Phaseolus*) should increase the level of understanding of the evolution of such specific antagonisms in a co-evolutionary framework. Finally, to confirm the results found in this first investigation of this important pest species a more complete sampling, not only of more specimens from the Neotropics, but also from the rest of the world where *A. obtectus* has spread, is needed (see also Alvarez et al., 2005).

ACKNOWLEDGEMENTS. We are very grateful to D. McKey, to one anonymous reviewer and to the editors of the *European Journal of Entomology* for discussion and for improving our English. We also thank A. Delgado-Salinas for providing information on the location of populations in Mexico and for providing Peruvian specimens. Finally, we thank C. Born, L. Mercier, J. Contreras and the team at the Instituto de Ecologia (UNAM) in Mexico City for discussion and logistic help. This

work was funded by the Swiss National Science Foundation (project N° 3100.064821.01) and the Centre d'Ecologie Fonctionnelle et Evolutive (CNRS).

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Received October 26, 2009; revised and accepted March 29, 2010