

Ecological distribution and niche segregation of sibling species: the case of bean beetles, *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell

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Abstract. 1. Molecular techniques have greatly added to the number of known sympatric cryptic species in insects. Ecological differences between these newly distinguished species are little explored, but niches often appear to overlap strongly. These cases are good models for exploring new ideas about species coexistence and community structure.

2. *Acanthoscelides obtectus* and *A. obvelatus* are two sister species of bean bruchids, which have been confused until the last decade. One important ecological difference between them has emerged, however: *A. obtectus* is multivoltine and now distributed worldwide, whereas *A. obvelatus* is univoltine and restricted to Mesoamerica. Where their ranges overlap, the two species share the same host plants and larvae can sometimes complete development in the same seed.

3. The analysis of 27 622 Mexican individuals of the two species in 2001–2002 and 2002–2003 indicates that their niches overlap, but are differentiated with respect to altitude and the kind of beans (wild vs. domesticated). The principal patterns in their relative abundance in different habitats, and at different seasons, were constant from one year to the next.

4. As sympatry of these species seems to be of recent origin, the observed niche differentiation may not have evolved in response to competition, but could instead be the consequence of physiological differences, evolved independently in each species in allopatry, that pre-adapted them for different altitudes and kinds of resources.

5. The combination of biological and historical factors thus appears to allow these two sibling species to coexist in sympatry, despite their broadly overlapping ecological niches.

Key words. *Acanthoscelides obtectus*, *Acanthoscelides obvelatus*, niche segregation, pest species, *Phaseolus*, sibling species.

Introduction

Mayr (1942) used the term ‘sibling species’ for the first time to designate species that cannot be diagnosed using a strictly morphological species definition. One of the first models of study of sibling species was the genus *Drosophila* (Diptera:

Drosophilidae), in which Dobzhansky (1946) first observed that morphologically almost identical species could coexist without inter-breeding. The emergence of comparative cytogenetic studies rapidly led to new insights in this genus (e.g. Burla *et al.*, 1949; Dobzhansky *et al.*, 1950; Salzano, 1956), in which sibling species often showed differences in arrangement and number of chromosomes. In *Drosophila*, the first attempts were made to examine ecological differentiation between sibling species. Sibling species seemed to occupy different habitats (Dobzhansky & Pavan, 1950; Da Cunha *et al.*, 1951; Da Cunha & Dobzhansky,

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1954; Sokoloff, 1955). In the late 1960s, with the advent of the study of allozyme polymorphism, new insights on the nature of sibling species emerged from work on *Anopheles* (Diptera: Culicidae) (Bianchi, 1968), and again in *Drosophila* (Hubby & Throckmorton, 1968; Ayala *et al.*, 1970; Ayala & Powell, 1972). With the emergence of techniques based on DNA analysis in the middle 1980s, new molecular tools confirmed the existence of additional sibling species in these two well-studied genera (in *Drosophila*: Stephens & Nei, 1985; Satta *et al.*, 1987; in *Anopheles*: Cockburn *et al.*, 1988). In the past two decades, numerous increasingly cryptic sibling species have been discovered (see Fig. 1) in various invertebrate groups (e.g. Sharpe *et al.*, 2000; Wilding *et al.*, 2000; Romi *et al.*, 2000; Navajas *et al.*, 2000; Hohenlohe & Boulding, 2001; Bond & Sierwald, 2002; Fettene *et al.*, 2002; Mattiucci *et al.*, 2003; Martin-Sanchez *et al.*, 2003; Maingon *et al.*, 2003; Wang *et al.*, 2003; Borghuis *et al.*, 2004; Duncan *et al.*, 2004; Schiffer *et al.*, 2004). It is likely that in some of these cases, more detailed morphological studies – limited by the global shortage of taxonomists – would uncover distinctive characters. The high discriminating power of contemporary molecular tools, however, allows more rapid discovery of sibling species, and for some highly cryptic sibling species molecular tools appear to supply the only reliable evidence (Hebert *et al.*, 2003, 2004).

Since the earliest studies on *Drosophila*, ecology and niche segregation of sibling species have been studied in about 25 genera of invertebrates. The niche can be defined according to

Hutchinson (1957; see also Silvertown, 2004) as an n-dimensional hypervolume defined by axes of resource use and/or environmental conditions, and within which populations of a species are able to maintain a long-term average net reproductive rate that is ≥ 1 . In insects, investigations on ecology and niche segregation of sibling species have continued to focus on the two well-studied genera *Anopheles* (e.g. White & Rosen, 1973; Mosha & Petrarca, 1983; Schneider *et al.*, 2000) and *Drosophila* (e.g. Yamamoto *et al.*, 1985; Aspi, 1996) but broadened to include other Diptera [e.g. *Rhagoletis* (Bush, 1969; Linn *et al.*, 2003), *Simulium* (Adler and Kim, 1984)], Lepidoptera [e.g. *Letha* (Shapiro & Carde, 1970), *Leptidea* (Benes *et al.*, 2003), *Astrartes* (Hebert *et al.*, 2004)], and Hymenoptera (e.g. *Asobara*; Vet *et al.*, 1984). In other invertebrates, ecological studies have focused on genera of copepods (Bergmans & Janssens, 1988), decapods (Walker, 2001), nematodes (Nascetti *et al.*, 1993), polychaetes (Gamenick *et al.*, 1998), and trematodes (Bartoli, 1988).

The number of behavioural, physiological, and morphological studies has kept pace with the discovery of new sibling species. The number of studies treating the ecology of sibling species has, however, not increased proportionally (see Fig. 1). Because most of these newly discovered sibling species were for long not distinguished from one another, their ecological differentiation is unstudied.

Niche differentiation therefore needs to be examined in some of the new cases. To some workers (e.g. Zhang *et al.*, 2004), the

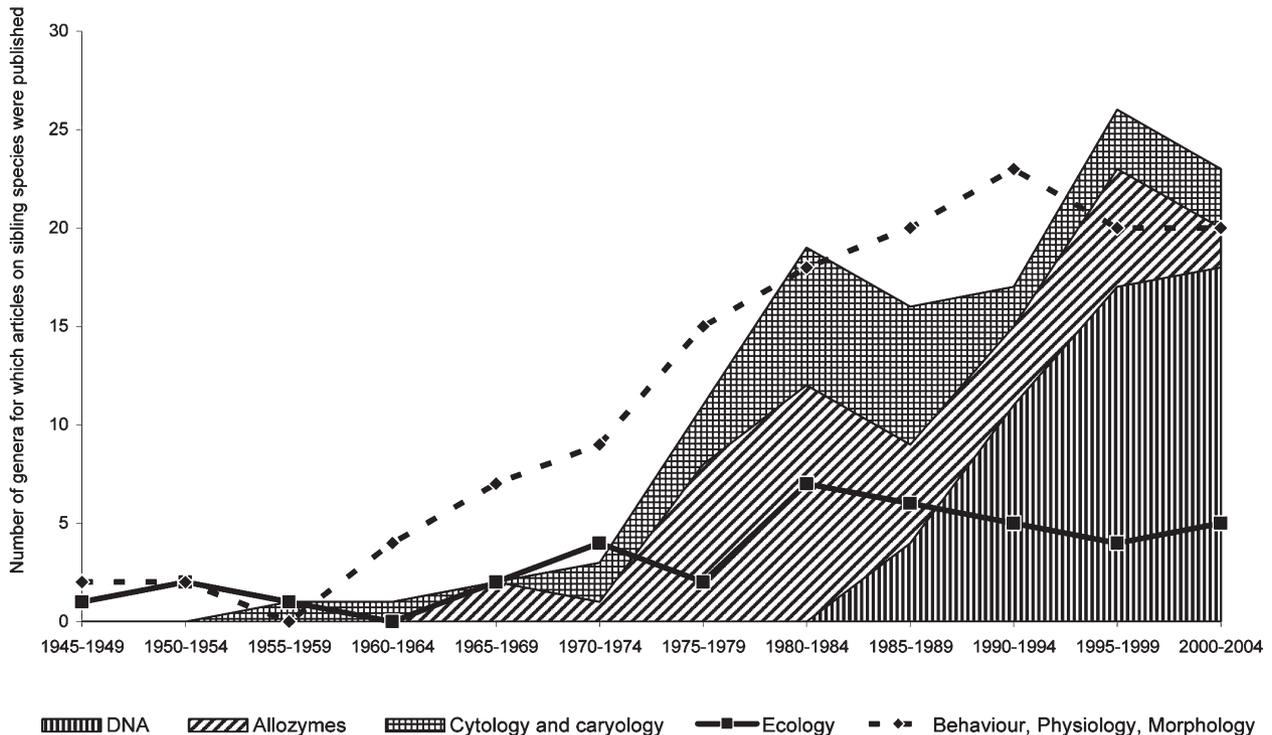


Fig. 1. Studies treating different aspects (ecology; DNA; cytology and caryology; allozymes; behaviour, physiology, and morphology) of sibling species, based on 298 publications on sibling species of invertebrates from 1945 to 2004 (source: Web of Science search using keyword 'sibling species'). The number of genera studied has been calculated for each period of time. Eighty-eight publications considered the genus *Drosophila*, whereas 70 treated *Anopheles*.

lack of obvious ecological segregation in sibling species suggests that these cases might provide support for Hubbell's (2001) neutral theory of community structure, which predicts that ecologically equivalent species can coexist for a long time, as dynamics of competitive exclusion or coexistence are slow for identical competitors. In an extreme case, such as the neotropical fig wasps in which Molbo *et al.* (2003) demonstrated several parallel cases of coexistence of supposedly morphologically identical sympatric species of pollinators in different fig species, the concept of niche segregation seems to be violated.

Niche segregation, however, could involve subtle differences, and fine-scale studies are required. Quantifying and understanding ecological differentiation between cryptic species is thus an emerging field of investigation. In such complex systems, information on how life-history traits can affect ecological segregation of different species has been little studied. The aim of this study is to compare the ecology of two sibling species of bruchid beetles (Coleoptera: Bruchidae), that are broadly sympatric and feed on the same larval hosts. Our study examines how a difference in a key life-history trait could lead to ecological segregation despite very broad niche overlap.

Bruchids are a well-studied family of seed-eating beetles, relatively homogeneous in terms of external morphological traits. About one-quarter of the species described belong to the neotropical genus *Acanthoscelides*, which comprises about 300 species (Johnson, 1983, 1990; Udayagiri & Wadhi, 1989). Among them, the great majority feed on legumes (Johnson, 1989). In this genus, many species are very similar in external morphology and can be distinguished only with great difficulty on the basis of morphological characters. This is particularly the case for two species associated with the common bean (*Phaseolus vulgaris*), *Acanthoscelides obvelatus* Bridwell and *A. obtectus* Say (Kingsolver, 1968). These two species belong to the same small clade within the genus, together with *A. argillaceus* Sharp, a Mesoamerican species specialised on beans of the *P. lunatus* L. group (Alvarez *et al.* 2006). Whereas *A. argillaceus* is morphologically distinct from the two others, *A. obtectus* and *A. obvelatus* have long been confused, because the only apparently diagnostic external morphological traits are the colour and shape of the last antennal segment. These diagnostic traits were recently confirmed by Alvarez *et al.* (2005a), although in a few cases, distinction between the two species was very difficult, requiring confirmation by examination of male genitalia. Despite their similarity, *A. obtectus* and *A. obvelatus* are two reproductively isolated species whose gene pools have accumulated substantial differences (Alvarez *et al.*, 2005a), confirming the validity of their separation by Kingsolver (1968).

In Mesoamerica, the two species often co-occur in the same habitats, on wild beans as well on domesticated beans. Leroi *et al.* (1990) suggested that *A. obtectus* was more adapted to the host plant *P. vulgaris* and *A. obvelatus* more adapted to *P. coccineus* L., another Mesoamerican species of the *P. vulgaris* group. Evidence for this hypothesis is weak, however. The functional trait that best differentiates the two species is their difference in voltinism (Biémont *et al.*, 1986). Whereas *A. obvelatus*, like most related species, is univoltine, *A. obtectus* is multivoltine. While multivoltinism has allowed *A. obtectus* to multiply in bean granaries, to exploit these continuously available resources, and to

move with beans during their introduction into new areas, multivoltinism is not an adaptation to bean domestication, because it pre-dated this event and characterises *A. obtectus* populations on wild beans as well (Alvarez *et al.*, 2005b). The extent to which differences in voltinism affect the ecology of these two bruchid species in natural populations in Mexico has not been studied.

To define more clearly the niches of these two bruchid species, their distribution in relation to several ecological factors was analysed, including not only the host-plant species identity, but also the altitude and the kind of beans (domesticated vs. wild), in a wide range of Mexican populations. In light of our results, the role of voltinism as the central factor explaining the patterns observed is also discussed.

Materials and methods

Sampling procedure

Forty-four sites ranging from latitude 18°49'03.6" to 19°50'56.6"N and from longitude 98°59'10.4" to 102°24'29.0"W were sampled in the Altiplano of southern Mexico between December 2001 and March 2002. Each site corresponds to a population of *Phaseolus* beans, either domesticated (23 sites), or wild (21 sites). Altitude and coordinates of each site are given in Appendix 1. Among the 23 sites for domesticated beans, 21 were *P. vulgaris* fields, while two were fields where *P. vulgaris* and *P. coccineus* were cultivated together. Of the 21 sites for wild beans, 12 were in wild *P. vulgaris* populations, five in wild *P. coccineus*, and four had wild populations of both *P. vulgaris* and *P. coccineus*. The two bean species demonstrate a similar seasonality, fruiting from December to April. For wild bean populations, mature pods were collected (between 100 and 1500, depending on the population size, with each pod containing between four and eight seeds), whereas for domesticated bean populations, farmers were asked to give (or sell) seeds of their last harvest (between 1 and 2 litres), except in rare cases where we were allowed to collect bean pods directly in the farmers' fields. Each site was sampled two times (rarely three), to capture the dynamics of the two species throughout the dry season, first at the beginning of the dry season (15 December 2001–15 January 2002), and a second time at the end of the dry season (15 February 2002–15 March 2002). During the first sampling period, bruchids cannot be detected by external visual examination. *Acanthoscelides* females start laying eggs in bean pods during the last stage of maturation of seeds (i.e. from early December to mid-January, depending on the population, in the Mexican Altiplano). Because *Acanthoscelides* need 30–80 days for development from eggs to adults in natural habitats (Labeyrie, 1962), all bruchids collected during the first sampling period were in a larval stage. In one single seed collection, however, different instars were present at the same time (N. Alvarez, pers. obs.). During the second sampling in the case of the multivoltine species *A. obtectus*, larvae of the second generation, stemming from eggs recently laid by adults of the first generation, were also present. In each collection site, pods were shelled and seeds placed in ventilated plastic recipients. Each day, emerging *A. obtectus* and *A. obvelatus* were collected in 70%

ethanol and the date of emergence was recorded. Adult beetles were collected daily to prevent them from reproducing in the plastic containers. Some reproduction may however, have been possible in the containers, as some emerging adults would have had about 24 h to copulate and lay eggs. In all sites with both *P. vulgaris* and *P. coccineus*, pods of the two species were separated, and the host plant from which each adult emerged was recorded. A third and final sampling was done between December 2002 and March 2003 in 16 of the 44 sampled sites, to examine inter-annual variation in the frequencies of the two species. In the first year, 14 537 individuals were collected, whereas in the second year, we collected a total of 13 085 individuals.

Statistical analysis

The effects of host-plant species, altitude, date of emergence and kind of host-plant population (domesticated vs. wild) on the respective proportions of individuals belonging to the two species were tested using a generalised linear model, with a binomial distribution and a logit link function (for each observation, an individual belonged either to *A. obvelatus* or to *A. obtectus*), using the 'genmod' procedure in SAS (1999). Because *P. coccineus* grows at higher altitudes and is much less frequently cultivated than *P. vulgaris*, the host-plant species could be a confounding factor of both the altitude and the cultivation status of the host. Correlation of host-plant species with altitude and with cultivation status (domesticated vs. wild) was therefore checked first, using the 'genmod' procedure in SAS. The effect of host plant was tested separately, by comparing the ratios of the number of *A. obvelatus* and *A. obtectus* individuals in the six mixed populations (where both *P. vulgaris* and *P. coccineus*

occurred) by a Wilcoxon signed rank test (based on paired comparisons), using S-plus, 2001). In these six populations, frequencies of *A. obvelatus* on *P. vulgaris* and on *P. coccineus* were compared by pairs. The effects of altitude, date of emergence and domestication status of the host-plant population (domesticated vs. wild) on the probability that a beetle would be *A. obvelatus* were then tested again – without taking into account the effect of host-plant species – using a generalised linear model. The estimates of the probability to be *A. obvelatus* for each of the modalities/variables were recorded for the three tested variables, and the probability distribution surface was drawn using S-Plus. The inter-annual variation was tested by a Wilcoxon signed rank test, using S-Plus, in which the frequencies of *A. obvelatus* in each site were compared for the 16 populations sampled in both years.

Results

The host-plant species (*P. vulgaris* vs. *P. coccineus*) ($\chi^2_1 = 10.45$, $P = 0.0012$), the domestication status (domesticated vs. wild) of host-plant ($\chi^2_1 = 938.00$, $P < 0.0001$), the altitude ($\chi^2_1 = 665.60$, $P < 0.0001$) and the date of emergence ($\chi^2_1 = 55.70$, $P < 0.0001$) all had effects on the relative frequencies of the two sibling species. It appears that host-plant species could be a confounding factor, as frequencies of the two host-plant species were strongly correlated with both domestication status ($\chi^2_1 = 1258.37$, $P < 0.0001$) and altitude ($\chi^2_1 = 4151.28$, $P < 0.0001$). In agreement with this interpretation, frequencies of *A. obtectus* and *A. obvelatus* on the two bean species in the mixed *Phaseolus* populations were not significantly different ($P = 0.5625$; see Fig. 2). Therefore, in a second analysis, the host-plant species

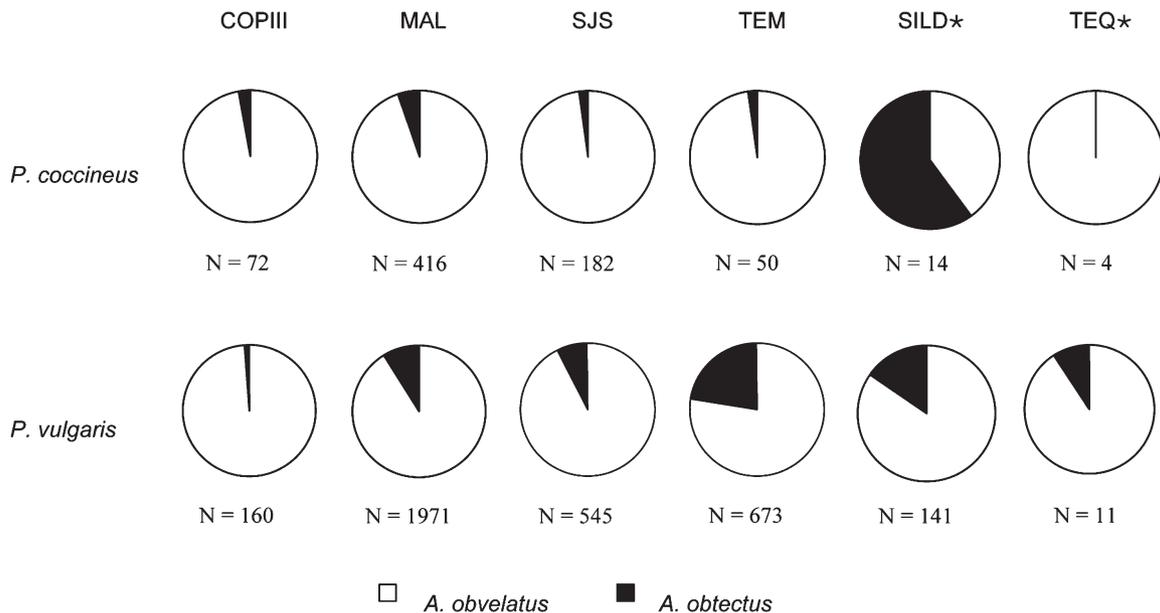


Fig. 2. Proportions of *A. obtectus* and *A. obvelatus* on *Phaseolus vulgaris* and *P. coccineus*, in six mixed populations where both bruchids were present. A '*' indicates a population of bruchids issued from domesticated beans. N is the number of emerging insects per population.

was not considered as a factor that might influence the frequencies of the two bruchids, and was simply discarded. In this analysis, frequencies of the two bruchids were examined in all populations, taking into account only domestication status, altitude, and date of emergence. The data did not show over-dispersion (scaled deviance = 0.7306). Again, each of the three factors showed a significant relationship with the frequencies of the two species: *A. obvelatus* was more frequent on wild than on domesticated beans ($\chi^2_1 = 1042.84$, $P < 0.0001$); it was more frequent at higher altitudes ($\chi^2_1 = 739.86$, $P < 0.0001$); and it became less frequent over the course of the reproductive season, from December to April ($\chi^2_1 = 62.53$, $P < 0.0001$). The probability surface of a sampled beetle being *A. obvelatus* (obtained from the 'genmod' estimates for each modality/factor) is represented in Fig. 3. In wild bean populations, *A. obvelatus* was never less frequent and almost always significantly more frequent than *A. obtectus*. Frequencies of *A. obvelatus* on wild beans ranged between 0.5 at low altitudes and 1.0 at high altitudes. On domesticated beans, *A. obvelatus* was more frequent than *A. obtectus* at higher altitudes, but less frequent at lower altitudes. Frequencies of *A. obvelatus* ranged between 0.15 (at low altitudes) and 1.0 (at high altitudes). Visual inspection of the data suggests that the 'boundary' between low-altitude populations, where *A. obtectus* was more frequent, and high-altitude populations, where *A. obvelatus* was more frequent, is situated at about 1800 m. Finally, the relative frequency of *A. obtectus* gradually increased between 15 December and 15 April. The comparison between 2002 and 2003 showed no significant differences in the proportions of the two bruchid species between years (paired Student's *t*-test, $t_{15} = -0.79$, $P = 0.44$; see Fig. 4).

Discussion

Niche segregation

The results show that although the niches of *A. obtectus* and *A. obvelatus* overlap broadly, they are statistically differentiated. *Acanthoscelides obvelatus* was more frequent at higher altitudes, and on wild bean populations, whereas *A. obtectus* was more common at lower altitudes and on domesticated beans. These two species are thus quite distinct ecologically. Other potentially discriminating dimensions of the bruchids' niches (e.g. parasitoid guilds), however, may segregate the two species as well, and the overall ecological distinctiveness of the two species may be even greater than indicated by our analysis. The importance of such unexplored dimensions of the respective niches of *A. obtectus* and *A. obvelatus* will be investigated in further studies.

The effect of altitude on the respective frequencies of the two *Acanthoscelides* sibling species depended on the domestication status of the host plant. Whereas on wild bean populations, the relative frequency of *A. obvelatus* decreased slowly with decreasing altitude, there was a much steeper decline in the relative frequency of this species with decreasing elevation on domesticated beans. This result indicates an interaction between the respective physiological aptitudes of the two species to evolve in cold/dry and warm/wet environments, and their

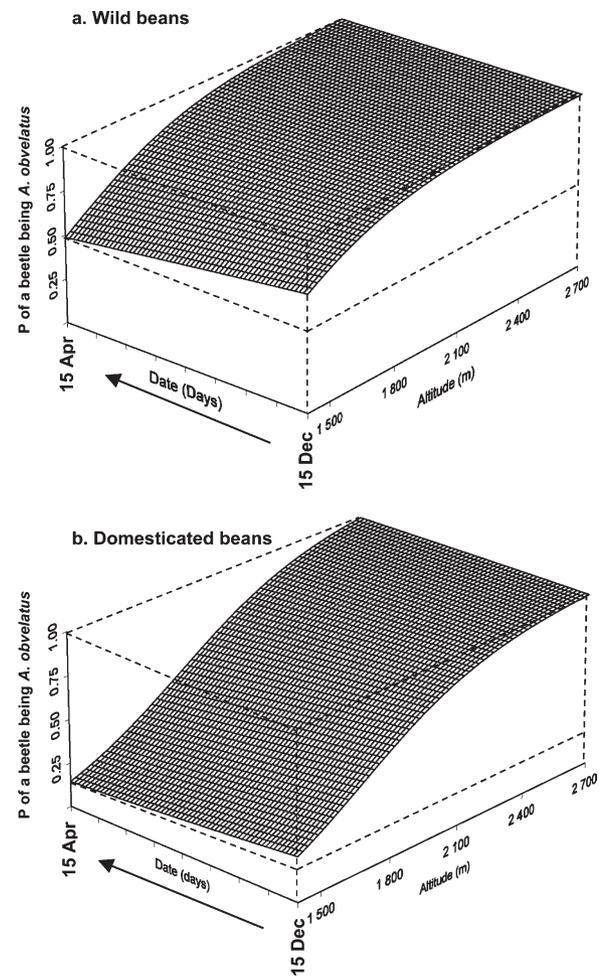


Fig. 3. Surface of the probability of a sampled beetle being *A. obvelatus*, as a function of altitude (from 1500 m to 2700 m) and the date of emergence (from 15 December to 15 April), for *Acanthoscelides obtectus* and *A. obvelatus* populations on (a) wild beans and (b) domesticated beans.

respective reproductive biologies. Because *A. obvelatus* only produces one generation per year, it is rapidly overtaken in numbers by *A. obtectus* in low-altitude granaries. Conversely, the impossibility for *A. obtectus* to enter reproductive diapause may impose severe limitations to its survival during the unfavourable season at higher altitudes. At such altitudes, the reproductive diapause of *A. obvelatus* (which is probably associated with a general reduction in metabolic activity) would confer higher survivorship, compensating for its ability to produce only a single generation per year. Temperature is likely to have a profound effect on the standard metabolic rate of heterotherms (Withers, 1992). Thus, as suggested by Janzen (1973), diapause in tropical insects should impose a severe energetic cost, because high temperature during the unfavourable season leads to high rates of respiration in heterothermous organisms such as insects, as well as high rates of water loss. The cost of reproductive diapause

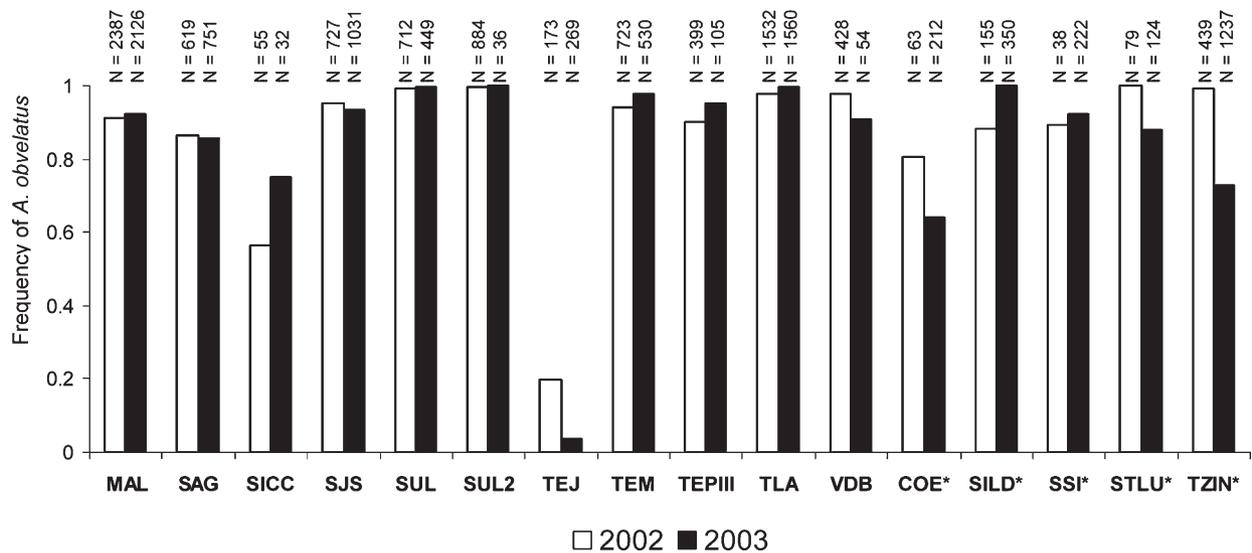


Fig. 4. Frequencies of *A. obvelatus* in years 2002 and 2003, for 16 populations of *Acanthoscelides obtectus* and *A. obvelatus*, in wild (11 populations) and domesticated beans (five populations). A '*' indicates a population of bruchids issued from domesticated beans. N is the number of emerging insects per population.

should therefore be lower at higher elevation, where temperatures in the unfavourable season are cooler than at lower elevation. This factor could partially contribute to explain the restriction of *A. obvelatus* to higher elevations.

In populations of wild beans, *A. obvelatus* was always more frequent than *A. obtectus*. This could be explained if wild beans always represent sink populations for *A. obtectus* (see Amarasekare *et al.*, 2004). The short adult lifetime of this species compared with *A. obvelatus* (Pichard *et al.*, 1991) coupled with the strong fruiting seasonality of wild *Phaseolus* beans in Mexico, may prevent its survival from one year to another.

Host-plant species effect

In opposition to the hypothesis of Leroi *et al.* (1990), our data from mixed bean populations show that the host-plant species (*P. vulgaris* vs. *P. coccineus*) did not affect the respective frequencies of the two sibling species of *Acanthoscelides*. The host-plant species is merely a factor that is confounded with the two other variables examined here, as in the Mexican Altiplano, *P. coccineus* grows at higher altitudes (1800–2700 m) than *P. vulgaris* (0–2100 m), and domesticated populations account for a low proportion of all populations of *P. coccineus*, in contrast to the situation for *P. vulgaris*.

Inter-annual stability of respective frequencies

Despite differences in the densities of populations of the two species from one year to another, the relative frequencies of *A. obvelatus* and *A. obtectus* remained similar in almost all populations, demonstrating the stability of their niche segregation. Assuming that both species have a relatively high dispersal

capacity (allowing each site to be repopulated each year with no continuity from the previous year's relative beetle densities at the same site), if niches were not well defined, a lottery situation would occur each year, leading to changes in relative frequencies from one year to the next in the different populations. While this result needs to be confirmed by longer-term studies, the observed stability of relative frequencies of each species over two successive years in virtually all populations is highly suggestive.

Role of voltinism

The effect of the date of emergence on the relative frequencies of the two species demonstrates the role played by voltinism. Whereas in *A. obvelatus* cohort overlapping is impossible, *A. obtectus* can produce multiple generations throughout the year as long as resources are available. Consequently, the relative frequency of *A. obtectus* gradually increased between 15 December and 15 April. Nevertheless, the higher frequency of *A. obvelatus* at the beginning of the season compared with its frequency 4 months later indicates a lower survival of *A. obtectus* during the rest of the year, as inter-annual frequencies are stable. This is possibly due to a higher mortality rate of *A. obtectus* during the rest of the season, especially in populations on wild beans, in which once the last bean pods have matured, resources are no longer available, and no further opportunities for oviposition occur within the lifetime of individuals. The highest longevity recorded for *A. obtectus* adults *in natura* is about 80 days (Labeyrie, 1962). Therefore, populations of this species on wild beans would appear to have a high probability of becoming locally extinct.

Even though multivoltinism has allowed *A. obtectus* to colonise granaries more easily than *A. obvelatus*, this trait is not the only one required for a bruchid to become a pest species. Indeed, both biological traits and historical accidents can moderate the

ability of a multivoltine bruchid species to develop in seed stocks. It is true that multivoltinism has allowed *A. obtectus* to become worldwide, but it has not resulted in this species becoming the most frequent species in Mesoamerica. Furthermore, the adaptation of *A. obvelatus* to altitude (i.e. its obligatory reproductive diapause, probably associated with a slower general metabolism) allows it to colonise bean populations where *A. obtectus* could not develop, independently of their domestication status. *Acanthoscelides obvelatus* is thus the only pest species present on domesticated beans at such altitudes. The combination of biological and historical factors therefore allows these two sibling species to coexist in sympatry, despite the similarity of several aspects of their niche.

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Appendix 1 List of sampled sites. A '*' besides the code indicates a population of bruchids issued from domesticated beans. The host-plant species is referred to as 'v' for *P. vulgaris* and as 'c' for *P. coccineus*.

Name	Code	Host-plant species	Latitude (°N)	Longitude (°W)	Altitude (m)
Arocutin	ARO*	v	19°33'22.5"	101°41'37.7"	2060
Coajomulco	COA	c	19°02'05.3"	99°12'00.3"	2664
Coatepec Harinas	COAT*	v	18°52'59.9"	99°46'00.1"	2100
Coeneo	COE*	v	19°49'13.9"	101°34'59.3"	2100
Coinzio	COI*	v	19°38'25.0"	101°16'28.2"	1905
Copandaro – campo	COP*	v	19°26'36.2"	101°46'27.3"	2120
Copandaro I	COPI	v	19°26'24.1"	101°45'35.5"	2087
Copandaro III	COPIII	v, c	19°26'26.1"	101°46'11.8"	2117
Erongaricuario	ERO	v	19°35'56.3"	101°42'32.8"	2072
Huitzilac	HUI	c	19°01'24.0"	99°16'23.3"	2544
Malinalco	MAL	v, c	18°57'13.2"	99°30'08.9"	1935
Napizarro	NAP*	v	19°35'50.5"	101°41'33.6"	2060
Ocumicho	OCU*	v	19°47'46.1"	102°13'11.8"	2045
San Andres de los Gabeles	SAG	v	19°02'19.5"	99°57'01.5"	2280
San Antonio	SAT*	v	19°38'49.9"	101°17'33.1"	1930
San Bartolo	SBO	c	19°14'31.7"	100°03'20.8"	2320
San Bartolo – campo	SBO*	v	19°14'29.8"	100°03'34.8"	2310
San Francisco Periban	SFP	v	19°32'31.4"	102°24'29.0"	1620
San Francisco Periban – campo I	SFPI*	v	19°32'33.5"	102°24'27.5"	1850
San Francisco Periban – campo II	SFPII*	v	19°32'32.4"	102°24'28.4"	1800
San Gabriel	SGA*	v	19°15'35.8"	100°07'28.1"	2292
San Isidro cerca Coeneo	SICC	v	19°50'56.6"	101°34'23.9"	2040
San Ildefonso	SILD*	v, c	19°22'19.8"	100°08'56.9"	2400
San Jose de los Laureles	SJS	v, c	18°58'49.7"	99°00'05.0"	1855
San Jose de los Laureles – campo I	SJS*	v	18°58'48.1"	98°59'35.0"	1800
San Jose de los Laureles – campo II	SJSCY*	v	18°58'40.3"	98°59'10.4"	1730
San Lorenzo	SLO*	v	19°31'33.3"	102°06'43.4"	2125
San Pedro de Tejalpa	SPTJ*	v	18°52'59.9"	99°36'00.0"	1750
San Simon	SSI*	v	19°01'27.4"	100°00'25.8"	2135
Santa Lucia	STLU*	v	18°52'12.5"	100°00'03.7"	1790
Santa Maria	STM*	v	18°49'03.6"	99°33'42.7"	2000
Sultepec I	SUL	v	18°51'07.8"	99°59'20.9"	2164
Sultepec II	SUL2	v	18°50'44.0"	99°58'04.1"	2200
Tejupilco	TEJ	v	18°55'51.2"	100°09'00.1"	1400
Tejupilco – campo	TEJ*	v	18°55'50.0"	100°09'00.3"	1400
Temascaltepec	TEM	v, c	19°02'35.9"	100°02'44.2"	1734
Tenango	TEN	c	19°06'38.7"	99°36'07.1"	2768
Tepoztlan I	TEPI	v	18°59'36.3"	99°07'15.7"	1931
Tepoztlan III	TEPIII	v	18°58'14.9"	99°07'19.2"	1700
Tequesquipan	TEQ*	v, c	19°03'09.2"	99°56'33.1"	2300
Tlalpan	TLA	c	19°17'50.3"	99°12'04.3"	2403
Tlayecapan	TLAY	v	18°57'20.0"	99°03'24.4"	1750
Tzintzuntzan	TZIN*	v	19°37'43.9"	101°34'41.5"	1980
Valle de Bravo	VDB	v	19°13'56.8"	100°07'05.1"	1918