Conservation Genetics of Potentially Endangered Mutualisms: Reduced Levels of Genetic Variation in Specialist versus Generalist Bees

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Abstract: *Oligolectic bees collect pollen from one or a few closely related species of plants, whereas polylectic bees visit a variety of flowers for pollen. Because of their more restricted range of host plants, it maybe expected that specialists exist in smaller, more isolated populations, with lower effective population sizes than generalists. Consequently, we hypothesized that oligolectic bees have reduced levels of genetic variation relative to related polylectic species. To test this hypothesis, we used five phylogenetically independent pairs of species in which one member was oligolectic and the other was polylectic. We assayed genetic variation in our species pairs at an average of 32 allozyme loci. Within each species pair, the oligolectic member had fewer polymorphic loci, lower average allelic richness, and lower average expected heterozygosity than its polylectic relative. Averaged over all species pairs, this corresponds to a 21% reduction in allelic richness, a 72% reduction in the proportion of polymorphic loci, and an 83% reduction in expected heterozygosity in specialists compared with generalists. Our data support the hypothesis of reduced effective population size in oligolectic bees and suggest that they may be more prone to extinction as a result. We suggest that in instances in which bee specialists are involved in mutually codependent relationships with their floral hosts, these mutualisms may be endangered for genetic and ecological reasons.*

Key Words: allelic richness, Apoidea, genetic variation, heterozygosity, Hymenoptera, oligolecty, pollination specialization

Genética de la Conservación de Mutualismos Potencialmente en Peligro: Bajos Niveles de Variación Genética en Abejas Especialistas Versus Generalistas

Resumen: *Abejas oligolécticas colectan polen de una o varias especies de plantas cercanamente relacionadas, mientras que abejas polilécticas visitan una variedad de flores para polen. Debido a que su rango de plantas huésped es más restringido, puede esperarse que las especialistas existan en poblaciones más pequeñas y más aisladas, con menor tamaño poblacional efectivo, que las generalistas. Consecuentemente, planteamos la hipótesis de que abejas oligolécticas tienen niveles reducidos de variación genética en relación con especies polilécticas. Para probar esta hipótesis utilizamos cinco pares de especies filogenéticamente independientes en los que un miembro era oligoléctico y otro poliléctico. Analizamos la variación genética en nuestros pares de especies en un promedio de 32 loci de alozimas. En cada par de especies, el miembro oligoléctico tenía menos loci polimórficos, menor promedio de riqueza alélica y menor promedio de heterocigosidad esperada que su pariente poliléctico. Promediado en todos los pares de especies, esto corresponde a una reducción de*

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21% de la riqueza alélica, reducción de 72% en la proporción de loci polimórficos y una reducción de 83% en la heterocigosidad esperada en especialistas comparados con generalistas. Nuestros datos soportan la hipótesis de reducción en el tamaño poblacional efectivo en abejas oligolécticas y sugieren que, como resultado, pueden estar más propensas a la extinción. Sugerimos que, en casos en que abejas especialistas están involucradas en relaciones mutuamente codependientes con sus huéspedes florales, estos mutualismos pueden estar en peligro por razones genéticas y ecológicas.

Palabras Clave: Apoidea, especialización en polinización, heterocigosidad, Hymenoptera, oligolectia, riqueza alélica, variación genética

Introduction

Pollinator declines will have major negative effects on terrestrial ecosystems throughout the world (Allen-Wardell et al. 1998). Oligolectic bees—which specialize on collecting pollen from only one or a few closely related plant species—make up a large proportion of the world's bee fauna (Michener 2000). They may surpass 60% of the noncleptoparasitic bee species, especially in arid environments (Moldenke 1979; Wcislo & Cane 1996). Although generalization has been hypothesized to be the norm in pollination systems (Waser et al. 1996), recent work has shown the opposite (Vázquez & Aizen 2003). In addition, specialist systems dominate certain habitats, geographic regions (Bond 1994; Michener 2000; Minckley et al. 2000), and taxonomic lineages (e.g., Ackerman 1983; Wcislo & Cane 1996; Sipes & Wolf 2001). The more tightly linked a particular pollinator is to its host flowering plant, the more susceptible each is to negative population trends in the other, suggesting that specialist plant-pollinator systems may be particularly vulnerable to extinction because of failed mutualisms (Bond 1994; Kearns et al. 1998). Consequently, understanding the conservation biology of oligolectic bees may be particularly important.

Despite its importance in conservation (Frankham 1995; Avise & Hamrick 1996), genetics is not commonly considered in insect conservation biology. In a recent survey of the conservation genetics of pollinators, Packer and Owen (2001) pointed out that genetic data for oligolectic bees are almost completely lacking. Indeed, the datum consisted of only one heterozygosity estimate for a species of *Macropis* (family Melittidae) oligolectic on *Lysimachia* (Pamilo et al. 1978). Since that survey, one detailed account of gene flow and population structure of an oligolectic bee has been published (Danforth et al. 2003). It is well known that small populations, including those of insects (Gilpin & Soulé 1986; Lacy 1993; Saccheri et al. 1998; Higgins & Lynch 2001), have a higher risk of extinction as a result of both genetic and stochastic events than do larger populations. Further, loss of genetic variation reduces the ability of species to adapt to environmental change (Fisher 1930; Frankham 1995) and is correlated with reduced population fitness (Hedrick & Kalinowski 2000; Reed & Frankham 2003) and metapopulation extinction (Saccheri et al. 1998). In addition, because sex in bees is determined by genotype at a hypervariable sex-determining locus, reduction in the effective population size (N_e) results in an increase in the proportion of homozygotes at this locus (Cook & Crozier 1995; Zayed & Packer 2001; Zayed et al. 2004). Such homozygous individuals are sterile diploid males, and their production increases the genetic load and decreases reproductive fitness (Page 1980; Ross & Fletcher 1986; Ross et al. 1993). In social species, diploid male production decreases colony growth rates and increases colony mortality (Plowright & Pallett 1979; Ross & Fletcher 1986). These factors suggest that bees are more prone to extinction for genetic reasons in comparison with other diploid pollinators.

Because of the more restricted range of host plants of specialist bees, it is expected that they persist in smaller, more isolated populations than do generalist bees. The resulting reduction in N_e, and thus in levels of neutral genetic variation (Crow & Kimura 1970; Frankham 1995), will likely render oligolectic bees and specialist pollination systems more prone to extinction. Studies of the population genetics of oligolectic bees, especially when directly compared with polylectic bees, are needed to elucidate the consequences of specialization on the conservation biology of bees. The purpose of this work was to test the hypothesis of reduced levels of genetic variation in specialist versus generalist bees.

Methods

Experimental Design and Sampling

We sampled bees for this study from the southern regions of the Atacama Desert in Chile because the area (1) is one of the least affected by humans in the world, which may have influenced bee populations; (2) has a high proportion of oligolectic bees (Arroyo et al. 1982); and (3) has a bee fauna that is comparatively well known (Toro 1986; Chiappa et al. 1990; Toro et al. 1996).

To test the hypothesis of reduced genetic variation in oligolectic versus polylectic bees, we collected samples

of five oligolectic and five polylectic bees for genetic analysis. To eliminate biases imposed by phylogenetic constraints on levels of genetic variation, we sampled phylogenetically independent pairs of species (Ridley 1983) in which one member was oligolectic and the other polylectic (Table 1). All pairs were phylogenetically independent in that each pair belonged to a different family or different subfamily within a family and in all cases the families or subfamilies were known to be monophyletic (Roig-Alsina & Michener 1993; Alexander & Michener 1995). Thus, each pair was strictly reciprocally monophyletic with respect to all others, thereby avoiding problems that may arise with less rigorous approaches to phylogenetic independence (Carpenter 1992).

Our strategy of replication over species pairs, spanning almost the entire phylogenetic diversity of bees, ensures that emerging trends were not largely influenced by patterns in any one species pair. We avoided confounding our analysis by sampling oligolectic species that are specialists on abundant flowers. In addition, we sampled them only from large populations, based on the knowledge of long-term field research of H.T. and L.R. Specialist bees with rare or restricted floral hosts were not sampled. *Loasa tricolor* Ker-Gawl, the floral host of *Caupolicana quadrifasciata* Friese and *Leioproctus rufiventris* (Spinola), was abundant with a large distribution from Regions II to VIII in Chile (Hoffmann 1995; Teillier et al. 1998). Similarly flowers in the genus *Nolana*, the host genus of *Nolanomelissa toroi* Rozen, were very abundant throughout Regions II to IV (Teillier et al. 1998; note that formal geographic subdivisions of Chile are numbered from I in the north to XIV in the south). *N. toroi* has been reported to collect pollen and nectar from *N. rostrata* (Lindl.) Dunal (Rozen 2003), which was abundant throughout Regions III and IV (Kohler 1970; Mesa 1981; Dillon & Hoffmann 1997). Cacti in the genus *Cumulopuntia*, the host of *Trichothurgus aterrimus* (Cockerell), were common in the Chilean altiplano in Regions I and II (Hoffmann 1989), and *Prosopis tamarugo* Phil., the host of *Centris mixta* Friese, existed in large populations in Regions I and II (Burkart 1976; Toro et al. 1993). Similarly, both specialist and generalist bees were native to Chile (i.e., none were introduced) and were sampled within their natural geographic distributions. *Caup. quadrifasciata* was found in Regions IV to VIII, *Cadeguala occidentalis* Haliday in Regions IV to X, *L. rufiventris* in Regions IV to VI, *Colletes seminitidus* Spinola in Regions I to X, *N. toroi* in Region III, *Acamptopoeum submetallicum* Spinola in Regions IV to IX, *T. aterrimus* (Cockerell) in Regions I and II, *Neofidelia longirostris* Rozen in Regions II and III, *Cen. mixta* in Regions I and II, and *Cen. chilensis* Spinola in Regions III to IX.

All bees were collected from flowers in the field during a single field season (September to December 2002). Each bee sample was collected during the 2 hours of peak flying activity, between 1200 and 1500 hours. Because the

Table 1. List of the taxa used to study differences in levels of genetic variation between specialists and generalist bee species.

Pair no.	*Oligolectic species*	*Locality (region)**	*Floral host*	*Polylectic species*	*Locality (region)**	*Family*	*Subfamily*
1	*Caupolicana quadrifasciata* Friese	Colliguay (IV)	*Loasa tricolor*	*Cadeguala occidentalis* Haliday	Colliguay (IV)	Colletidae	Diphaglossinae
2	*Leioproctus rufiventris* Spinola	Colliguay (IV)	*Loasa tricolor*	*Colletes seminitidus* Spinola	Colliguay (IV)	Colletidae	Colletinae
3	*Nolanomelissa toroi* Rozen	Vallenar (III)	*Nolana rostrata*	*Acamptopoeum submetallicum* Spinola	Fray Jorge (IV)	Andrenidae	Panurginae
4	*Trichothurgus aterrimus* (Cockerell)	San Pedro (II)	*Cumulopuntia* sp.	*Neofidelia longirostris* Rozen	North of Chañaral (II)	Megachilidae	Lithurginae and Fideliinae, respectively
5	*Centris mixta* Friese	San Pedro (II)	*Prosopis tamarugo*	*Centris chilensis* Spinola	Llanos de Challe (III)	Apidae	Anthophorinae

**Roman numerals refer to formal geographic subdivisions of Chile, which are numbered from I in the north to XIV in the south.*

collection effort was similar for all samples, the sample size of each species could be used to indicate relative abundance, which did not significantly differ for oligolectic versus polylectic bees (paired t test: $t = -0.4923$, df $= 4$, two-tailed $p = 0.648$). Bees were killed by immersion in liquid nitrogen, where they remained until they were transported from Chile to York University (Toronto, Canada) on dry ice. In the laboratory they were stored in an ultracold freezer at -80° C. Voucher specimens for all sampled species were deposited in the Packer collection at York University and in the collection of the Pontificia Universidad Católica, Valparaíso, Chile.

Electrophoresis and Genetic Analysis

We used allozyme electrophoresis on starch gels to assay genetic variation following standard protocols (Packer & Owen 1989; Packer & Owen 1990). We attempted to resolve the same suite of 30 enzyme-staining systems for all taxa. Some systems, however, did not produce scorable bands for some species. We scored an average of 32 loci per species. Enzyme names and abbreviations were as follows: 6-phosphogluconate dehydrogenase (6PGD), aspartate aminotransferase (AAT), adenylate kinase (AK), aldolase (ALD), aldehyde dehydrogenase (ALDDH), arginine kinase (ARK), diaphorase (DIA), esterase (EST), fumarase (FUM), glycerol-3-phosphate dehydrogenase (G3PDH), glucose-6-phosphate dehydrogenase (G6PDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glucosephosphate isomerase (GPI), hydroxybutyric dehydrogenase (HAD), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), octanol dehydrogenase (ODH), peptidase gly-leu (PEPGL), peptidase leu-ala (PEPLA), peptidase n-acetyl methionine (PEPM), peptidase phe-pro (PEPPP), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH), and superoxide dismutase (SOD). Data on the number of allozyme loci scored for each species, along with allele frequencies for the variable loci, are available at http://www.yorku.ca/bugsrus/olipolygenvar.htm.

We used three parameters to quantify genetic variation: (1) proportion of loci that are polymorphic, P, regardless of allele frequencies; (2) unbiased expected heterozygosity, H_{exp}, estimated following Nei (1978); and (3) allelic richness, R, estimated following El Mousadik and Petit (1996) as implemented in FSTAT (version 2.9.3, Goudet 1995). Allelic richness is a measure of the number of alleles at a locus corrected for variation in sample size. Because FSTAT requires that data be entered as diploid genotypes, genotype data from haploid males were merged to form diploid genotypes when males were sampled. This has no effect on the allelic richness estimates because the method uses allelic frequency data and not genotypic data. Both H_{exp} and R were averaged over loci for each species. We compared our average H_{exp} data with those from previously published studies of solitary noncleptoparasitic bees (Packer & Owen 2001) from the same families as our species pairs, where the number of loci surveyed was ≥ 15, as required for estimates of H_{exp} to be meaningful (Graur 1985).

Data from allozyme loci tend to follow expectations of the neutral theory of molecular evolution (Kimura 1983; Skibinsk & Ward 1998; Crochet 2000). Under the infinite-allele model (IAM) of mutation (Kimura & Crow 1964), the equilibrium heterozygosity, H_e, is

$$H_e = \frac{4N_e u}{4N_e u + 1}, \tag{1}$$

where u is the mutation rate. Solving for N_e yields

$$N_e = -\frac{H_e}{4u(H_e - 1)}. \tag{2}$$

By assuming that mutation rates for protein-coding loci are equal within closely related species pairs (Kimura 1983; but see Rodriguez-Trelles et al. 2001), the relative reduction in N_e of oligolectic species in comparison to their polylectic relative can be represented as the following ratio:

$$\frac{N_{e.o}}{N_{e.p}} = \frac{-H_{e.o}\left(H_{e.p} - 1\right)}{-H_{e.p}(H_{e.o} - 1)}, \tag{3}$$

where o and p refer to oligolectic and polylectic parameters respectively. We used Eq. 3 to investigate the relative differences in N_e between specialists and generalists. We assumed that species were at their equilibrium heterozygosity where $H_e = H_{\text{exp}}$. Effective population size can also be derived from H_e under the stepwise-mutation model (Ohta & Kimura 1973). Our prior analysis, however, revealed that both mutation models yielded essentially identical results given our data set; thus, for brevity, we report estimates of N_e ratios only under IAM. The calculation of N_e based on heterozygosity data involved several assumptions, some of which are unlikely to be met in the taxa we were studying. Consequently, we did not perform statistical analyses on the presented ratios which were provided only as a simple means of showing the magnitude of the difference in N_e between pair members.

Statistical Analysis

We used both paired-sample t tests and sign tests (Zar 1999) to examine differences between P, average H_{exp}, and average R estimates in each of the phylogenetically independent oligolectic-polylectic pairs. We tested the null hypothesis of no difference in levels of genetic variation between oligolectic and polylectic bees against the alternative hypothesis of reduced levels of genetic variation in the former versus the latter. Our alternative hypothesis has strong theoretical backing (reviewed in Futuyma & Moreno 1988), and the opposite pattern has not received

Table 2. Levels of genetic variation between specialist and generalist bees, measured as the proportion of polymorphic loci (*P*), average allelic richness (*R*), and average expected heterozygosity (H_{exp}).

	Bee species pair number[a]									
	1a	*1b*	*2a*	*2b*	*3a*	*3b*	*4a*	*4b*	*5a*	*5b*
P (%)	6.45	54.84	15.15	26.67	2.94	26.09	19.05	25.00	5.71	41.94
R^b	1.06 (0.045)	1.63 (0.124)	1.14 (0.059)	1.29 (0.096)	1.03 (0.029)	1.39 (0.150)	1.21 (0.068)	1.25 (0.078)	1.05 (0.033)	1.35 (0.086)
$H_{exp}{}^b$	0.014 (0.012)	0.076 (0.023)	0.006 (0.003)	0.060 (0.026)	0.002 (0.002)	0.046 (0.023)	0.010 (0.003)	0.027 (0.013)	0.015 (0.014)	0.071 (0.023)
$N_{e.o}/N_{e.p}{}^c$ (%)	17.26	–	9.46	–	4.16	–	36.40	–	19.93	–
n^d	22	32	31	34	32	30	32	30	32	29

[a] *Species pairs, as presented in Table 1, end with (a) for specialist and (b) for generalist.*
[b] *Standard errors (SE) are provided in parentheses.*
[c] *The effective population size of the specialist species ($N_{e.o}$) represented as a ratio of the effective population size of its generalist pair member ($N_{e.p}$).*
[d] *Number of diploid genotypes sampled.*

any empirical support from studies on a wide range of phytophagous insects (Peterson & Denno 1998*a,b*). Because we hypothesized that specialists have lower levels of genetic variation than generalists, we report only one-tailed *p* values for our statistical tests.

Results

Genetic variation, measured as the proportion of polymorphic loci, *P*, average allelic richness, *R*, and average expected heterozygosity, H_{exp}, was lower in the oligolectic species in all our paired comparisons (Table 2). Oligolectic bees had, on average, a 21% reduction in allelic richness, 83% lower average expected heterozygosity, and 72% fewer polymorphic loci. The pattern of lower genetic variation in oligolectic versus polylectic species was statistically significant (paired *t* tests, *P*: $t = -3.205$, $df = 4$, $p = 0.016$; *R*: $t = -3.123$, $df = 4$, $p = 0.017$; H_{exp}: $t = -5.863$, $df = 4$, $p = 0.002$; sign tests for *P, R,* H_{exp}: $p = 0.031$). The N_e of oligolectic bees, represented as a fraction of the N_e of polylectic bees, averaged 17% across all species pairs (Table 2). For two species pairs (2 and 3), the oligolectic member's N_e was an order of magnitude lower than that of the polylectic member.

In comparison with previously published data on solitary noncleptoparasitic bees from the same families (Table 3), our oligolectic species had the lowest H_{exp}. The exception was the Andrenidae, for which there was one zero value that was below the very low levels we recorded for our oligolectic andrenids. In contrast, our polylectic species had H_{exp} estimates that were within the ranges of or above those reported for other taxa.

Discussion

It is well known that, in comparison with other insects, the Hymenoptera have reduced levels of genetic variation (Metcalf et al. 1975; Pamilo et al. 1978) as do the haplodiploid Thysanoptera (Crespi 1991). That the reduced effective population size resulting from haplodiploidy is at least partially responsible for this seems well founded (Hedrick & Parker 1997; Packer & Owen 2001). What is less readily explained is why bees have heterozygosity levels that are even further reduced than those of other Hymenoptera (Packer & Owen 2001). Data available at the time of Packer and Owen's (2001) survey were insufficient to permit a comparison between oligolectic and polylectic bee species. Our results indicate that oligolectic bees have reduced levels of genetic variation in comparison to polyleges, thereby demonstrating that they have particularly low levels of genetic variation, even for Hymenoptera.

Several lines of evidence suggest that specialist bees exist in smaller, more isolated populations than generalist bees. Results of studies of a wide range of insects show that the density of the plant host is strongly correlated with the density of its insect herbivores (Kery et al. 2001 and references therein). If the population density of polylectic bees is controlled by the density of several floral hosts, it should be higher on average than the population density of oligolectic bees, whose density is dependent on a single host, all other factors being equal. Additionally, there is a clear relationship between spacing of floral hosts and bee flight distance (e.g., Levin & Kerster 1969; Schulke & Waser 2001) such that pollinators are less abundant in isolated patches of flowers. In metapopulations, gene flow plays an important role in increasing N_e because it counteracts the effects of extinction-recolonization dynamics responsible for reductions in heterozygosity in subpopulations (Hedrick & Gilpin 1997). Subdivided populations, with no or low gene flow, have lower N_e than panmictic populations with similar population sizes (Hedrick & Gilpin 1997; Whitlock & Barton 1997). Thus, isolation alone can be responsible for the reduction of N_e in oligolectic versus polylectic bees.

Table 3. Comparisons of expected heterozygosity estimates between our studied bees and those available for confamilial solitary species.

Family/species	*Pollen collection method*[a]	H_{exp}	*Confamilial rank in* H^b_{exp}	*Reference*
Colletidae				
Leioproctus rufiventris (Spinola)	oligo	0.006	1	herein
Caupolicana quadrifasciata Friese	oligo	0.014	2	herein
Colletes seminitidus (Spinola)	poly	0.060	3	herein
Colletes succinctus (L.)	poly	0.064	4	Pamilo et al. 1978
Cadeguala occidentalis (Haliday)	poly	0.076	5	herein
Andrenidae				
Nolanomelissa toroi Rozen	oglio	0.002	2	herein
Andrena vaga Panzer	poly	0.000	1	Pamilo et al. 1978
Andrena lapponica Zetterstedt	poly	0.007	3	Pamilo et al. 1978
Andrena wilkella (Kirby)	poly	0.037	4	Pamilo et al. 1978
Acamptopoeum submetallicum (Spinola)	poly	0.046	5	herein
Megachilidae				
Trichothurgus aterrimus (Cockerell)	oligo	0.010	1	herein
Megachile pacifica Panzer	poly	0.015	2	Lester & Selander 1979
Neofidelia longirostris Rozen	poly	0.027	3	herein
Megachile inermis Provancher	poly	0.045	4	Packer et al. 1995
Megachile relativa Cresson	poly	0.050	5	Packer et al. 1995
Megachile rotundata (Fab.)	poly	0.062	6	McCorquodale & Owen 1997
Apidae				
Centris mixta Friese	oligo	0.015	1	herein
Svastra sp.	poly	0.038	2	Lester & Selander 1997
Centris chilensis (Spinola)	poly	0.071	3	herein

[a] *Oligolectic (oligo) bees collect pollen from one or a few closely related species of plants, and polylectic (poly) bees visit a variety of flowers for pollen.*
[b] *Ranked from lowest to highest.*

In a study of isolation by distance (IBD) in phytophagous insects, monophagous species had higher proportions of significant IBD relationships and smaller *y*-intercept values on graphs of IBD than polyphagous species (Peterson & Denno 1998*a*). The differences between the two groups, however, were not statistically significant, possibly because of the small number of strictly monophagous species studied (Peterson & Denno 1998*a*). The former result suggests that gene flow is more restricted in specialists than in generalists. Additionally, the neighborhood size, analogous to N_e, is approximated by 10^b, where b is the y intercept on the IBD graph (Slatkin 1993). Thus, the latter finding of Peterson and Denno (1998*a*) suggests that specialists have lower N_e.

Our data demonstrate reduced levels of genetic variation in oligolectic bees, which supports the hypothesis of reduced effective population sizes of the former versus the latter. This, in turn, suggests that specialists are at a higher risk of endangerment or extinction for genetic and demographic reasons and less likely to be able to adapt to changing environmental conditions (Soulé 1980; Frankel & Soulé 1981; Gilpin & Soulé 1986; Frankham 1995; Avise & Hamrick 1996; Frankham et al. 2002). In addition, low N_e in bees results in elevated levels of sterile diploid male production (Zayed & Packer 2001; Zayed et al. 2004). Sterile diploid males impose a heavy genetic load and severely reduce reproductive fitness, increasing the risk of extinction in specialist populations. Depending on the extent to which their floral hosts actually rely on their oligolectic bees for pollination, our data suggest that such mutualisms might also be endangered.

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