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Increased genetic differentiation in a specialist versus a generalist bee: implications for conservation

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Abstract

Oligolectic bees are specialists that collect pollen from one or a few closely related species of plants, while polylectic bees are generalists that collect pollen from both related and unrelated species of plants. Because of their more restricted range of floral hosts, it is expected that specialists persist in more isolated populations than do generalists. We present data on the population structure of two closely related bee species sampled from a super abundant floral host in the southern Atacama Desert. Pairwise comparisons of population subdivision over identical distances revealed that the specialist bee had significantly more differentiated populations in comparison to the generalist. Further, populations of the specialist had significantly less genetic variation, measured as observed and expected heterozgyosity, than those of the generalist. Our data support the hypothesis of decreased gene flow among populations of the specialist bee even at equivalent geographic distances. The resulting reductions in effective population size for specialists make them particularly prone to extinction due to both demographic and genetic reasons. Our findings have important implications for the conservation of bees and other specialist insects.

Introduction

Bees are integral and indispensable components of almost all terrestrial ecosystems, and their global conservation is of prime importance (Allen-Wardell et al. 1998). Bees can be broadly classified into two functional groups; oligolectic species collect pollen from only one or a few closely related plant species (i.e. specialists), while polylectic species collect pollen from many plant species (i.e. generalists) (Cane and Sipes 2006). Oligolectic bees make up a significant proportion of the world's bee fauna: 15–30% of all pollen collecting bee species in temperate and subtropical regions, and 43–60% in xeric environments (Minckley and Roulston 2006), where bee species richness is usually highest (Michener 2000). Understanding the conservation biology of oligolectic bees is therefore essential for attempting to maintain the worldwide biodiversity of bees, and for conserving specialized flower-pollinator mutualisms (Bond 1994; Kearns et al. 1998). Further, recently posited global declines of pollination services are suggestive of large reductions in bee populations (Kevan and Viana 2004), stressing the need for understanding the conservation biology of these ecologically and economically important pollinators.

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A recent survey of the conservation genetics of pollinators (Packer and Owen 2001) highlighted the paucity of pertinent data for oligolectic bees and noted that comparative studies between oligolectic and polylectic species had never been undertaken. Comparative data for specialists and generalists are needed to clarify the role of specialization in increasing extinction risk, and for testing hypotheses of the evolution of specialization. As a consequence of specialization, the geographic distribution of oligolectic bees will be limited by that of their floral host(s), while polylectic bees should be able to maintain higher levels of gene flow through areas in which the host of a specialist species is absent. Thus, populations of specialists are expected to be more isolated than those of generalists (reviewed by Futuyma and Moreno 1988). Isolation will result in a reduction of gene flow between populations, and a reduction in effective population size (N_e) (Hedrick and Gilpin 1997; Whitlock and Barton 1997), ultimately resulting in reduced levels of neutral genetic variation (Kimura 1983). If specialization results in isolation, then specialist species should have reduced levels of neutral genetic variation when compared to generalist species. We recently tested this hypothesis by comparing levels of genetic variation at allozyme loci in five phylogenetically independent oligolectic – polylectic species pairs (Packer et al. 2005). We found that specialist species had significantly lower levels of genetic variation (measured as % polymorphic loci, average allelic richness, and average expected heterozygosity) than did generalists. Based on the magnitude of the reduction of genetic variation in specialist bees, we estimated that N_e of specialists averaged nearly an order of magnitude lower than that of generalists (Packer et al. 2005), supporting the hypothesis of increased isolation in specialists.

In the only study of population structure of oligolectic bees, Danforth et al. (2003) found significant levels of population subdivision in *Macrotera* (*Macroteropsis*) *portalis* (Andrenidae) in mixed Chihuahuan desert habitats in Southeastern Arizona, USA. Population differentiation in *M. portalis* ranked in the highest 10% among 92 species of insects surveyed for that parameter (Danforth et al. 2003). However, without comparative data from related generalist bees, it is impossible to conclude whether the high population subdivision in *M. portalis* is due to oligolecty *per se*, patchiness of its xeric habitat, or some other factor. Here we present an analysis of the population structure of a closely related oligolectic – polylectic species pair occurring in sympatry in the southern Atacama Desert in Chile to test the hypothesis of greater isolation between populations of specialists versus generalists.

The two species that we compare are from the same subfamily, the Colletinae, of the family Colletidae. *Leioproctus rufiventris* (Spinola) (Colletidae), is a specialist on *Loasa tricolor* Ker-Gawl (Loasaceae), one of the most widespread and abundant flowers in central Chile (Teillier et al. 1998). This bee is known from throughout the range of its floral host: regions IV–VI (Packer et al. 2005). Given that other species of Loasaceae in this region are uncommon (Teillier et al. 1998), and we have not observed *L. rufiventris* on them, it seems very likely that this species is strictly monolectic (following Cane and Sipes 2006). On the other hand, *Colletes seminitidus* Spinola (Colletidae) is polylectic, occurring from Regions I–X, beyond the range of *Lo. tricolor*. However, *C. seminitidus* exhibits floral constancy upon *Lo. tricolor* where this plant is common (i.e. females mainly collect pollen only from *Lo. tricolor*, where its bloom is abundant). However, in areas outside the range of this plant, and in localities within its distributional range where it is not found, such as in urban areas, *C. seminitidus* can be found on a wide range of alternative floral hosts. Such floral constancy is common, if not typical, within populations of generalist species (Minckley and Roulston 2006). This allowed us to sample both species on the same floral host from the same localities, and to conduct pairwise comparisons of genetic differentiation over identical distances, removing the confounding effect of distance on gene flow and levels of genetic differentiation (Peterson and Denno 1998). Further, both species are solitary ground nesting bees with similar body sizes, expected to fall in the ‘‘moderately mobile’’ dispersal category of Peterson and Denno (1998) based on average home range sizes for solitary bees (Cane 2001). The phylogenetic and ecological similarities between these two species should make the degree of floral specialization the major influence upon any observed differences in estimates of genetic differentiation between their respective populations.

Methods

Sampling

Bees were sampled on *Lo. tricolor* from five localities within a 2-h period during peak flying activity (between 12 and 3 pm) from September 25 to October 13 2002 (Table 1 and Figure 1). Bees were killed by immersion in liquid nitrogen where they remained until transportation from Chile to York University (Toronto ON, Canada) on dry ice. Upon arrival in Toronto, samples were stored in an ultracold freezer at −80 °C until used for electrophoresis. Voucher specimens for both species are housed in the Packer collection at York University, Toronto, Canada and the collection of the Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

Allozyme electrophoresis

Both *L. rufiventris* and *C. seminitidus*, sampled from Tongoy (Table 1) were previously assayed for genetic variation at 33 and 29 allozyme loci respectively (Packer et al. 2005). *L. rufiventris* was polymorphic at Adenylate kinase (AK), Esterase (EST, 2 loci), Hexokinase (HK), and Malate dehydrogenase (MDH), while *C. seminitidus* was polymorphic at Aldehyde dehydrogenase (ALDDH), Arginine kinase (ARK), Diaphorase (DIA), EST, Fumarase (FUM), HK, MDH, and Malic enzyme (ME). For analysis of population structure, we assayed all additional samples of both species for variation at these polymorphic loci. During the screening process, we detected allelic variants for ME in *L. rufiventris* – LR4, originally monomorphic at LR1. We scored ME for all *L. rufiventris* samples, and included it in our analysis.

Data analyses

We examined population structure using Wright's *F* statistics (Wright 1951; Weir and Cockerham 1984), as implemented in FSTAT 2.9.3 (Goudet 1995). Significance of *F* statistics was estimated using 1000 randomizations in FSTAT. Pairwise F_{ST} between all population pairs was also estimated using FSTAT. F_{ST} is a robust measure of genetic differentiation (Whitlock and McCauley 1999; Neigel 2002) and we used it to compare levels of genetic differentiation between populations of specialists and generalists.

By sampling both generalists and specialists at exactly the same localities, we removed the confounding effect of distance from our analyses of genetic differentiation (Peterson and Denno 1998; Packer and Owen 2001). Although intraspecific comparisons of pairwise F_{ST} values violates the assumption of independence in standard parametric and nonparametric statistical tests, differences in pairwise F_{ST} estimates between the two species over the same distances are statistically independent (e.g. Felsenstein 1985). We used the Wilcoxon matched-pairs signed-ranks method (Zar 1999) to test the null hypothesis of zero median difference between pairwise F_{ST} for *L. rufiventris* and *C. seminitidus* (indicating no difference in levels of genetic differentiation over identical distances), versus the alternative hypothesis of a positive median difference (indicating greater levels of genetic differentiation in *L. rufiventris* over identical distances). We also examined the extent of isolation by distance (IBD) between the two species by regressing pairwise F_{ST}, versus the logarithm of pairwise distance (D) (Rousset 1997), using reduced major axis (RMA) regression, the significance of which was tested

Table 1. Sampling localities and diploid sample sizes (n) for the generalist *C. seminitidus*, and the specialist *L. rufiventris*

Sampling locality	*C. seminitidus*		*L. rufiventris*	
	Sample name	Size	Sample name	Size
Tongoy, 30°15.25′ S 71°30.30′ W	CS1	32	LR1	26
Near Los Hornos, 29°39.32′ S 71′18.05′ W	CS2	32	LR2	31
Quebrada Seca, 30′33.41′ S 71′26.52′ W	CS3	33	LR3	12
Parque Nacional Fray Jorge, 30°40.53′ S 71°38.16′ W	CS4	33	LR4	26
Near Socos, 30°41.71′ S 71°28.73′ W	CS5	33	LR5	19

All samples were obtained from Chile, Region IV.

using a Mantel test (1000 randomizations) as implemented in IBD 1.4 (Bohonak 2002).

We used *t*-tests to compare average observed heterozygosity (H_o), and Nei's unbiased expected heterozygosity (H_{exp}) (Nei 1978), estimated using POPGENE 1.32 (Yeh and Boyle 1997), between subpopulations of *L. rufiventris* and *C. seminitidus*. Under the infinite-allele model (IAM) of mutation (Kimura and 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 the closely related *L. rufiventris* and *C. seminitidus* (Kimura 1983, but see Rodriguez-Trelles et al. 2001), the relative reduction in N_e of the former in comparisons to the latter can be represented as the following ratio:

$$\frac{N_{e\cdot o}}{N_{e\cdot p}} = \frac{-H_{e\cdot o}(H_{e\cdot p} - 1)}{-H_{e\cdot p}(H_{e\cdot o} - 1)} \tag{3}$$

where the subscripts o and p refer to oligolectic and polylectic parameters, respectively. We investigated the relative differences in N_e between *L. rufiventris* and *C. seminitidus* by using equation 3 where $H_e = H_{exp}$ as estimated for each species and population. We did not perform statistical analysis upon N_e estimates, and only presented ratios as a simple means of showing the magnitude of the difference in N_e between the specialist and generalist bee.

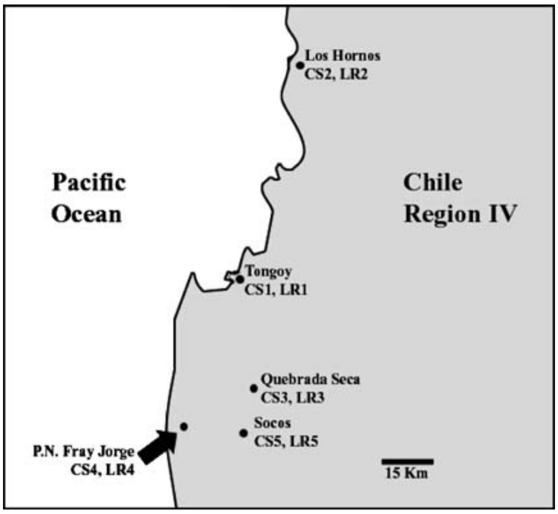

Figure 1. Map of Chile (Region IV) showing sampling locations for the generalist bee *C. seminitidus* (CS), and the specialist bee *L. rufiventris* (LR).

Results

Allele frequencies for both species are presented in Table 2. *L. rufiventris* had significantly lower observed and expected heterozygosity than *C. seminitidus* (Table 3. observed heterozygosity: $t = 6.014$, df = 8, $P < 0.001$; expected heterozygosity: $t = 7.116$, df = 18, $P < 0.001$). On average, *L. rufiventris* populations exhibited an 83% reduction in N_e when compared to those of *C. seminitidus* (Table 3). Significant heterozygote deficiencies, measured as F_{IS}, were found in both species (*C. seminitidus*: $F_{IS} = 0.195$; *L. rufiventris*: $F_{IS} = 0.230$; $P < 0.001$ for both tests).

Significant population subdivision was found in both species (*L. rufiventris*: $F_{ST} = 0.68$. *C. seminitidus*: $F_{ST} = 0.254$; $P < 0.001$ for both tests), with the specialist having a global F_{ST} estimate more than twice as large as that for the generalist species. Estimates of pairwise F_{ST} between populations of the specialist were significantly higher than those of the generalist when compared over similar distances (Figure 2; Wilcoxon matched-pairs signed-ranks test: $W+ = 51$, $W- = 4$, $n = 10$, one-tailed $P = 0.0068$). On average, this represented more than a two fold increase (207%) in pairwise F_{ST} of *L. rufiventris* over *C. seminitidus*. Both species showed patterns of IBD that approached statistical significance (Figure 3: for the specialist: $y = 1.03x–1.21$, $r^2 = 31\%$, $P = 0.094$; for the generalist: $y = 0.42x–0.46$, $r^2 = 18\%$, $P = 0.063$), with pairwise F_{ST} appearing to increase at a faster rate over distance in the specialist versus the generalist species. However, the lack of significance (at $\alpha = 0.05$) of both IBD regressions render statistical testing for difference in regression slopes meaningless.

Discussion

Our findings show that populations of the specialist bee exhibited higher levels of genetic differentiation in comparison to those of the

Table 2. Allele frequencies, and diploid sample sizes, n, for *C. seminitidus* (CS) and *L. rufiventris* (LR) populations.

Locus		CS1	CS2	CS3	CS4	CS5
ALDDH	1	0.969	0.859	0.939	0.939	1
	2	0.031	0.141	0.061	0.061	0
	n	32	32	33	33	33
ARK	1	0.726	0.667	0.015	0.000	0.061
	2	0.274	0.333	0.985	1.000	0.939
	n	31	15	33	33	33
DIA	1	0.859	0.969	0.879	0.97	0.833
	2	0.141	0.031	0.121	0.03	0.167
	n	32	32	33	33	33
EST	1	0.953	0.875	0.227	0.439	0
	2	0.047	0.125	0.773	0.561	1
	n	32	32	33	33	33
FUM	1	0	0	0	0	0.03
	2	0.281	0.641	0.576	0.621	0.561
	3	0.484	0.266	0.318	0.227	0.364
	4	0.234	0.094	0.106	0.152	0.045
	n	32	32	33	33	33
HK	1	0.016	0.161	0.061	0.091	0.061
	2	0.984	0.645	0.848	0.364	0.939
	3	0	0.194	0.061	0.379	0
	4	0	0	0.03	0.167	0
	n	32	31	33	33	33
MDH	1	0	0.031	0.152	0.106	0.045
	2	0.125	0.25	0.091	0.152	0.273
	3	0.875	0.719	0.758	0.742	0.682
	n	32	32	33	33	33
ME	1	0	0	0.015	0	0.045
	2	1	0.813	0.788	0.939	0.924
	3	0	0.188	0.197	0.061	0.03
	n	32	32	33	33	33
		LR1	LR2	LR3	LR4	LR5
AK	1	0.981	1	0	0	0
	2	0.019	0	1	0.962	1
	3	0	0	0	0.038	0
	n	26	31	12	26	19
EST-1	1	0.019	0	0.083	0	0
	2	0.981	0.79	0.208	0.404	0
	3	0	0.21	0.708	0.596	1
	n	26	31	12	26	19
EST-2	1	0.038	0.032	0	0.038	0
	2	0.962	0.968	1	0.962	1
	n	26	31	12	26	19
HK	1	1	1	1	1	0.974
	2	0	0	0	0	0.026
	n	26	31	12	26	19
MDH	1	0.019	0.065	0	0	0
	2	0	0	0.125	0	0.026
	3	0.981	0.935	0.875	1	0.974
	n	26	31	12	26	19
ME	1	0	0	0	0.038	0
	2	1	1	1	0.962	1
	n	26	31	12	26	19

Table 3. Observed (H_o) and expected heterozygosity (H_{exp}) in populations of the generalist *C. seminitidus* (CS) and the specialist *L. rufiventris* (LR)

Sample	H_o	H_{exp}	Sample	H_o	H_{exp}	$N_{e \cdot o}/N_{e \cdot p}$ (%)
CS1	0.1769	0.2088	LR1	0.0165	0.0273	11
CS2	0.2555	0.3401	LR2	0.0507	0.0747	16
CS3	0.2386	0.2839	LR3	0.0714	0.0994	28
CS4	0.2955	0.3027	LR4	0.0714	0.1025	26
CS5	0.1477	0.2072	LR5	0.0150	0.0150	6
Mean	0.2223	0.2685		0.0450	0.0638	17%
SD	0.0599	0.0589		0.0280	0.0406	10%

The effective population size of the specialist ($N_{e \cdot o}$) is represented as a ratio to that of the generalist ($N_{e \cdot p}$) for each locality.

generalist bee. Although both species showed significant population structure, most likely attributable to the patchy nature of floral resources in their xeric environment (e.g. Danforth et al. 2003), populations of the specialist bee showed higher levels of genetic differentiation when compared to the generalist (Figure 2). Reduced levels of genetic differentiation between populations of *C. seminitidus*, when compared with *L. rufiventris*, most likely represent additional dispersal opportunities available to the generalist through the utilization of other floral hosts between populations of *Lo. tricolor*. The significant reduction in H_{exp}, and H_o in populations of *L. rufiventris* versus those of *C. seminitidus* are consistent with our earlier study comparing levels of neutral genetic variation in species pairs of oligolectic and polylectic bees (Packer et al. 2005), supporting the hypothesis of reduced N_e in specialists. Although IBD regressions only approached statistical significance (Figure 2), we attribute this to the low statistical power for detecting IBD in our small dataset; a minimum of 15 populations is usually needed to detect significant IBD (Peterson and Denno 1998). Interestingly, both specialist and generalist had significant F_{IS} values as were found in *M. portalis* (Danforth et al. 2003). This is surprising given that a proportion of *M. portalis* males are flightless and do not vacate their natal nest, implying a potential to inbreed. It is not know whether our studied species actively seek matings with related individuals. It may be possible that high F_{IS} is a general phenomenon in solitary bee populations, which tend to exist in patchy viscous populations (Packer and Owen 2001), the effects of which might be especially pronounced in already patchy xeric habitats.

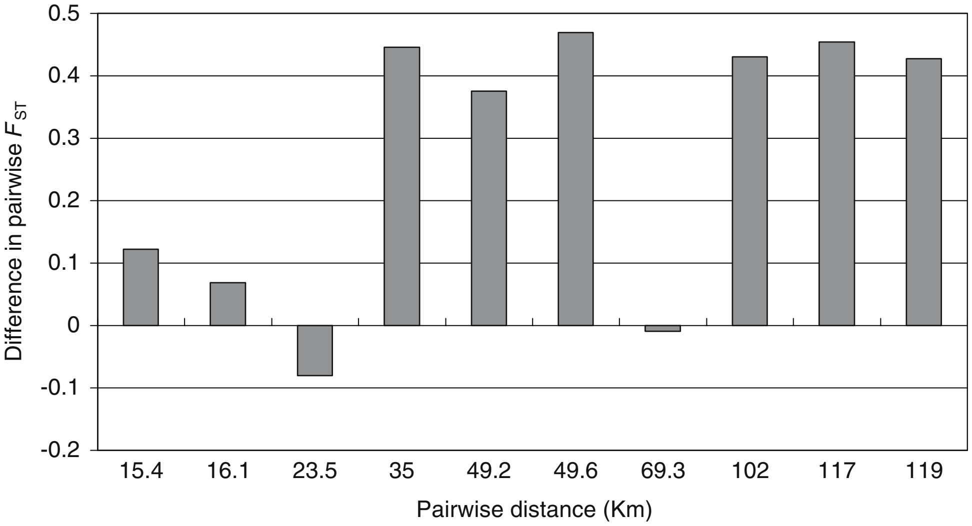

Figure 2. Difference in pairwise F_{ST} between populations of the specialist and the generalist (i.e. F_{ST} of specialist – F_{ST} of generalist) compared over the same distances. Positive values indicate more genetic differentiation in specialists versus generalists, and *vice versa*.

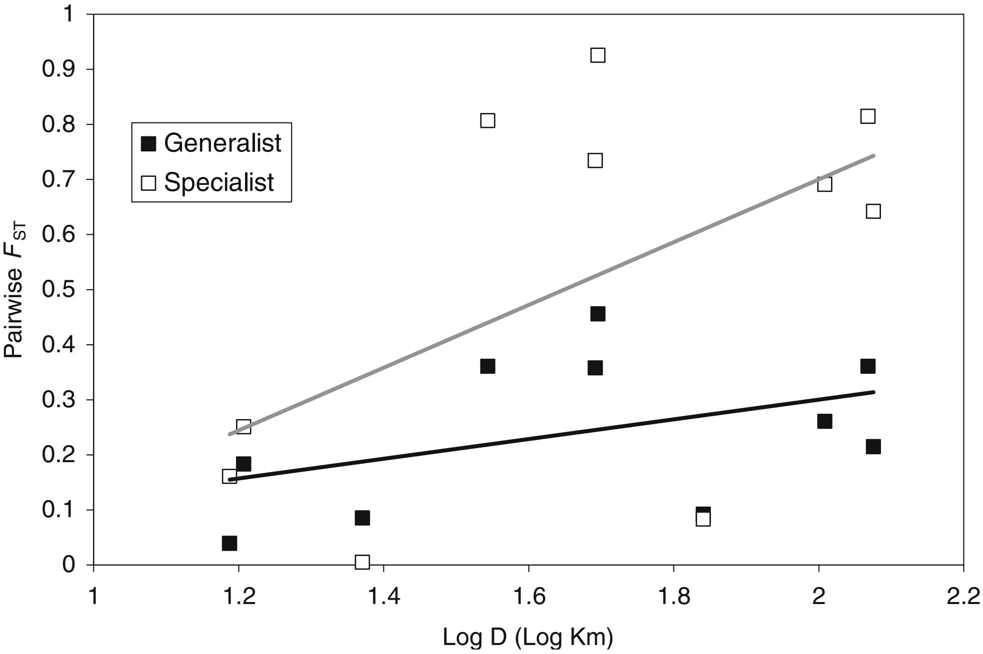

Figure 3. Regression between pairwise genetic differentiation, measured as F_{ST} and pairwise distance (D) for populations of the specialist and generalist bee.

More pairwise comparisons of population structure in closely related taxa would likely provide a clearer perspective on the possible consequences of specialization on the population genetics of specialist insects. Kelley et al. (2000) found stronger patterns of genetic differentiation in a specialist bark beetle versus its generalist sister species, consistent with our findings. Kelley et al. also found lower levels of genetic variation at mitochondrial DNA in the specialist versus the generalist. In a study of population structure differences between two sister species of milkweed beetles, Dobler and Farrell (1999) found stronger levels of geographic structure in populations of the generalist versus the specialist, however, this might be explained by differences in dispersal abilities between the two species, where the specialist was more vagile. In a meta-analysis of 43 phytophagous insects, Peterson and Denno (1998) showed that diet breadth did not influence isolation by distance patterns. However, the definition of monophagous used by Peterson and Denno (feeding on one plant genus) may not reflect strong specialization (Kelley et al. 2000; Cane and Sipes, 2006), possibly biasing their conclusions. Peterson and Denno's (1998) results, although not statistically significant, indicated that a higher proportion of monophagous insects exhibited significant IBD patterns than polyphagous insects. This lack of significance might be due to statistical noise in the dataset generated from pooling data over many taxa regardless of phylogenetic relationships (Carpenter 1992), and through errors arising from variable reporting of data inherent in meta-analysis methodologies (Lajeunesse and Forbes, 2003).

Our results shed some light on the pattern of increased speciation rates in specialist lineages, and the unusual biodiversity patterns of bees worldwide. It is well documented that most specialist species are members of ancestrally specialist lineages, both for animals in general (reviewed by Futuyma and Moreno 1988) and in bees (Wcislo and Cane 1996), and such lineages often specialize on the same host (reviewed by Futuyma and Moreno 1988; Sipes and Wolf 2001; Minckley and Roulston 2006). If our findings, and those of Kelley et al. (2000), prove to be the norm in specialist populations, then faster speciation in specialist lineages could simply be an artifact of rapid evolution in small isolated populations (reviewed by Barton and Charlesworth 1984; Howard 1993). Rapid allopatric and/or allochronic speciation in specialist lineages in patchy xeric habitats is consistent with the finding that bees attain their highest diversity in such environments (Linsley 1958), where a large proportion of the bee fauna is oligolectic (Minckley and Roulston 2006), and where specialization seems to confer the greatest fitness benefits over generalization through better host-synchrony (Minckley and Roulston 2006).

We would like to note that in, some aspects, the effects of habitat fragmentation on bee populations should be similar to those induced by diet specialization, as both specialization and fragmentation promote population isolation. It is thus expected that fragmented bee populations suffer from loss of genetic variation and reduced N_e. High dispersal abilities can counteract the effects of fragmentation, and it is sometimes presumed that, due to their sometimes large foraging ranges, bees are good dispersers and are thus tolerant of fragmentation. Mixed findings on the effects of fragmentation on bee populations (reviewed by Cane 2001), help propagate this view. We would like to dispel such presumptions on the following grounds. First, foraging distances should not be taken to represent dispersal ability, and more importantly gene flow (for definitions of dispersal, migration, and gene flow, see Loxdale and Lushai 1999). The fact that a bee travels a specific distance per day while in the process of foraging does not imply gene flow over the same distance or even that nest sites average similar distances apart from one generation to the next. For example, orchid bees have very large home ranges and are capable of flying long distances (Roubik 1989) but recent work has shown that gene flow among their populations is restricted (Zayed et al. 2004). Second, both theoretical arguments (haplodiploidy, nest building, central place foraging, philopatry), and empirical evidence suggest reduced gene flow in bee populations when compared to diploid insects (Packer and Owen 2001). Finally, since studies on the effects of fragmentation on bee populations have ignored genetic aspects, processes of great consequence to the long term sustainability of bee populations might have gone unstudied (Zayed et al. 2004).

Specialization has been previously correlated with extinction proneness (Bond 1994; Labandeira et al. 2002; Koh et al. 2004). For example, Kotiaho et al. (2005) found narrow diet specialization to be a common ecological characteristic of threatened Finnish butterflies. In addition to their susceptibility to extinction due to failed mutualisms (i.e. through the extinction of host plants, Koh et al. 2004), our data suggest that specialist bees are more prone to extinction than generalists, for intrinsic reasons: They exist in more isolated populations with lower N_e. Populations with small effective sizes have a higher risk of extinction due to both genetic and stochastic events than do larger populations (Frankham 1995; Frankham et al. 2002), and loss of genetic variation in small populations reduces adaptability (Fisher 1930; Frankham et al. 2002) and is correlated with metapopulation extinction (Saccheri et al. 1998). Further, loss of genetic diversity in haplodiploid bees represents an additional threat as it increases levels of diploid male production (Cook and Crozier 1995; Zayed and Packer 2001; Zayed et al. 2004). Diploid males are either inviable or effectively sterile, and their production increases the genetic load and decreases reproductive fitness (Cook and Crozier 1995), reduces effective breeding population sizes (Zayed 2004), and dramatically increases extinction risk (Zayed and Packer 2005). Specialist bees are thus expected to be more extinction prone than generalist bees. This is corroborated by the finding that habitat fragmentation dramatically affects ground nesting specialist bees, but not generalists (Cane et al. cited in Cane 2001), indicative of higher extirpation rates for the former. Similarly, specialization in diet (Goulson et al. 2005), and habitat requirements (Williams et al. 2005) have been linked to rarity and declines in British bumblebees. Also, higher extinction rates for specialist phytophagous insects during the terminal Cretaceous event have been inferred from the fossil record (Labandeira et al. 2002). Specialist bees, representing a significant proportion of the world's bee fauna, are thus expected to be more adversely affected by anthropogenically-induced changes to their environments than are generalist bees.

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References

Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann SL, Cane JH, Cox PA, Dalton V, Feinsinger P, Ingram M, Inouye DW, Jones CE, Kennedy K, Kevan PG, Koopowitz H, Medellin R, Medellin-Morales S, Nabhan GP (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conserv. Biol.*, **12**, 8–17.

Barton NH, Charlesworth B (1984) Genetic revolutions, founder effects, and speciation. *Annu. Rev. Ecol. Syst.*, **15**, 133–164.

Bohonak AJ (2002) IBD (Isolation By Distance): A program for analyses of isolation by distance. *J. Hered.*, **93**, 154–155.

Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and dispersal disruption on plant extinction. *Philos. Trans. R. Soc. Lond. Ser. B*, **344**, 83–90.

Cane JH (2001) Habitat fragmentation and native bees: A premature verdict? *Conserv. Ecol.*, **5**, 3 [online] URL: http://www.consecol.org/vol5/iss1/art3/index.html.

Cane JH, Sipes SD (2006) Characterizing floral specialization by bees: Analytical methods and a revised lexicon for oligolecty. In: *Specialization and Generalization in Plant–Pollinator Interactions* (eds. Waser NM, Ollerton J), In press. University of Chicago Press, Chicago, USA.

Carpenter JM (1992) Comparing methods. *Cladistics*, **8** 191–196.

Cook JM, Crozier RH (1995) Sex determination and population biology of the Hymenoptera. *Trends Ecol. Evol.*, **10**, 281–286.

Danforth BN, Ji S, Ballard LJ (2003) Gene flow and population structure in an oligolectic desert bee, Macrotera (Macroteropsis) portalis (Hymenoptera: Andrenidae). *J. Kans. Entomol. Soc.*, **76**, 221–235.

Dobler S, Farrell BD (1999) Host use evolution in *Chrysochus* milkweed beetles: Evidence frm behaviour, population genetics and phylogeny. *Mol. Ecol.*, **8**, 1297–1307.

Felsenstein J (1985) Phylogenies and the comparative method. *Am. Nat.*, **125**, 1–15.

Fisher RA (1930) *The Genetic Theory of Natural Selection*, Dover, New York.

Frankham R (1995) Conservation genetics. *Annu. Rev. Genet.*, **29**, 305–327.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge.

Futuyma DJ, Moreno G (1988) The evolution of ecological specialization. *Annu Rev. Ecol. Syst.*, **19**, 207–233.

Goudet J (1995) FSTAT, version 1.2; a computer program to calculate F statistics. *J. Hered.*, **86**, 485–486.

Goulson D, Hanley ME, Darvill B, Ellis JS, Knight ME (2005) Causes of rarity in bumblebees. *Biol. Conserv.*, **122**, 1–8.

Hedrick PW, Gilpin ME (1997) Genetic effective size of a metapopulation. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds. Hanski IA, Gilpin ME), pp. 165–181. Academic Press Inc., San Diego, USA.

Howard DJ (1993) Small populations, inbreeding, and speciation In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (eds. Thornhill NW), pp. 118–142. The University of Chicago Press, Chicago USA.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant–pollinator interactions. *Annu. Rev. Ecol. Syst.*, **29**, 83–112.

Kelley ST, Farrell BD, Mitton JB (2000) Effect of specialization on genetic differentiation in sister species of bark beetles. *Heredity*, **84**, 218–227.

Kevan PG, Viana BF (2004) The global decline of pollination services. *Biodiversity*, **4**, 3–8.

Kimura M (1983) *The Neutral Theory of Molecular Evolution*, Cambridge University Press, Cambridge, UK.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725–738.

Koh LP, Dunn RR, Sodhi NS, Colwell RK, Proctor HC, Smith VS (2004) Species co-extinctions and the biodiversity crisis. *Science*, **305**, 1632–1634.

Kotiaho JS, Kaitala V, Komonen A, Päivinen J (2005) Predicting the risk of extinction from ecological characteristics. *Proc. Natl. Acad. Sci. USA*, **102**, 1963–1967.

Labandeira CC, Johnson KR, Wilf P (2002) Impact of the terminal Cretaceous event on plant–insect associations. *Proc. Natl. Acad. Sci. USA*, **99**, 2061–2066.

Lajeunesse MJ, Forbes MR (2003) Variable reporting and quantitative reviews: a comparison of three meta-analytical techniques. *Ecol. Lett.*, **6**, 448–454.

Linsley EG (1958) The ecology of solitary bees. *Hilgardia*, **27**, 543–599.

Loxdale HD, Lushai G (1999) Slaves of the environment: The movement of herbivorous insects in relation to their ecology and genotype. *Philos. Trans. R. Soc. Lond. Ser. B*, **354**, 1479–1495.

Michener CD (2000) *The Bees of the World*, The Johns Hopkins University Press, Baltimore, Maryland.

Minckley RL, Roulston TH (2006) Incidental mutualisms and pollen specialization among bees. In: *Specialization and Generalization in Plant-Pollinator Interactions* (eds. Waser NM, Ollerton J), pp. 69–98. The University of Chicago Press, Chicago, USA.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Neigel JE (2002) Is Fst obsolete? *Conserv. Genet.*, **3**, 167–173.

Packer L, Owen R (2001) Population genetic aspects of pollinator decline. *Conserv. Ecol.*, **5**, 4 [online] URL: http://www.consecol.org/vol5/iss1/art4.

Packer L, Zayed A, Grixti JC, Ruz L, Owen RE, Vivallo F, Toro H (2005) Conservation genetics of potentially endangered mutualisms: Reduced levels of genetic variation in specialist versus generalist bees. *Conserv. Biol.*, **19**, 195–202.

Peterson MA, Denno RF (1998) The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *Am. Nat.*, **152**, 428–446.

Rodriguez-Trelles F, Tarrio R, Ayala FJ (2001) Erratic overdispersion of three molecular clocks: GPDH, SOD, and XDH. *Proc. Natl. Acad. Sci. USA*, **98**, 11405–11410.

Roubik DW (1989) *Ecology And Natural History Of Tropical Bees*, Cambridge University Press, Cambridge.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Sipes SD, Wolf PG (2001) Phylogenetic relationships within Diadasia, a group of specialist bees. *Mol. Phylo. Evol.*, **19**, 144–156.

Teillier S, Zepeda H, García P (1998) *Flores del Desierto de Chile*, Marisa Cuneo Ediciones, Valdivia, Chile.

Wcislo WT, Cane JH (1996) Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annu. Rev. Entomol.*, **41**, 257–286.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. *Genetics*, **146**, 427–441.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: Fst does not equal 1/(4Nm + 1). *Heredity*, **82**, 117–125.

Williams P (2005) Does specialization explain rarity and decline among British bumblebees? A response to Goulson et al. *Biol. Conserv.*, **122**, 33–43.

Wright S (1951) The genetical structure of populations. *Ann. Eugen.*, **15**, 323–354.

Yeh FC, Boyle TJB (1997) Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg. J. Bot.*, **129**, 157.

Zar JH (1999) *Biostatistical Analysis*, Prentice-Hall, Upper Saddle River, New Jersey.

Zayed A (2004) Effective population size in Hymenoptera with complimentary sex determination. *Heredity*, **93**, 627–630.

Zayed A, Packer L (2001) High levels of diploid male production in a primitively eusocial bee (Hymenoptera: Halictidae). *Heredity*, **87**, 631–636.

Zayed A, Packer L (2005) Complementary sex determination substantially increases extinction proneness of haplodiploid populations. *Proc. Natl. Acad. Sci. USA*, **102**, 10742–10746.

Zayed A, Roubik DW, Packer L (2004) Use of diploid male frequency data as an indicator of pollinator decline. *Proc. Roy. Soc. Lond. Ser. B (Suppl.)*, **271**, S9–S12.