SHORT COMMUNICATION

Variable assortative mating in replicate mating trials using *Drosophila melanogaster* populations derived from contrasting opposing slopes of 'Evolution Canyon', Israel

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Abstract

Significant assortative mating in laboratory studies has been previously shown between two populations of *Drosophila melanogaster* collected from micro-climatically contrasting and opposing slopes of 'Evolution Canyon' (Lower Nahal Oren, Israel; Korol *et al.*, 2000). Coupled with evidence that the two populations are adapted to their respective environments, this has been suggested as a rare example of ongoing behaviourally mediated speciation occurring in the face of gene flow. Reproductive isolation between these populations, however, has never been confirmed by replicate experiments in an independent laboratory. For this reason, we tested recent collections of these populations for premating isolation in both the original (Haifa) and a new (Burnaby) laboratory under a variety of experimental protocols. Although non-random mating was found in the majority of trials conducted in Haifa, we were unable to replicate these strong results in Burnaby. Most notably, we failed to detect assortative mating in four separate double choice experiments. Significant non-random mating was detected, however, in three of six single choice experiments in Burnaby, suggesting that the populations are behaviourally differentiated in some manner. Why nonrandom mating was weaker in Burnaby than Haifa is not understood, but suggests that assortative mating may be sensitive to unknown environmental factors.

Introduction

It has been suggested that the divergence of *Drosophila melanogaster* inhabiting the opposing, and micro-climatically differing, slopes of 'Evolution Canyon' represents an early stage in parapatric ecological speciation, whereby divergent natural selection drives the accumulation of genetic differences among the populations that generate reproductive isolation (Korol *et al.*, 2000; Iliadi *et al.*, 2001). The differing temperature and available moisture between the south- and north-facing sides of the canyon (which exhibit 'African' and 'European' biotas,

respectively; see Nevo, 1995) are hypothesized to exert strong, divergent selection on the flies inhabiting each slope (Korol *et al.*, 2000). Differential selection on various ecological traits, including behaviour, is hypothesized to have produced some behavioural isolation as a by-product, thus driving their incipient speciation despite the fact that the close proximity (100–400 m) of the populations should permit dispersal and subsequent gene flow (Korol *et al.*, 2000). In support of this, adaptive differences between the populations have been documented and include: larvae viability under various temperatures (Rashkovetsky *et al.*, 2000), oviposition temperature preferences, resistance of adults to drought stress (Nevo *et al.*, 1998), mating propensity, egg laying schedules (Iliadi *et al.*, 2001), and even rates of recombination (see, e.g. Derzhavets *et al.*, 1996). In addition, genetic differences in the frequency of an insertion in the regulatory region of hsp70Ba (a major inducible heat shock protein) have been reported (Michalak *et al.*, 2001).

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Further genetic analysis, however, has produced mixed results. Synthetic laboratory populations from each slope (each established with 25 isofemale lines collected in 1997 and kept under random mating for 50–55 generations) show significant differentiation across microsatellite loci (Michalak *et al.*, 2001); however, analyses of wild flies collected at the same time do not (Colson, 2002; Schlötterer & Agis, 2002). Additionally, no significant between-slope differentiation was found across six accessory gland protein (*Acp*) loci in isofemale lines established in the lab in 1995 (Panhuis *et al.*, 2003). The extent of genetic differentiation between the populations in nature is thus unclear.

Mate choice experiments using populations collected in 1995 and 1997 (and kept for 28 and 48 generations, respectively, in constant laboratory conditions) have shown a strong preference by both sexes for mates from their own slope (Korol *et al.*, 2000). This is consistent with models of parapatric ecological speciation in which reproductive isolation evolves in the face of gene flow due to disruptive natural selection (Schluter, 2000; Via, 2001; Coyne & Orr, 2004). A systematic analysis of sexual behaviour of these flies by Iliadi and colleagues indicated that females from the north-facing slope discriminated strongly against males of the opposite slope, and both sexes from the south-facing slope displayed distinct reproductive and behavioural patterns when compared with north-facing slope individuals. These included, in females, increased fecundity, shorter time before remating, and relatively higher receptivity, and in males, higher mating propensity (Iliadi *et al.*, 2001).

Recently, Panhuis *et al.* (2003) conducted a limited set of double choice mating trials (males and females from both slopes mixed in a single cage), using the 1995 lines, and failed to find any assortative mating. These contradictory findings suggest further investigation is needed. We conducted extensive mate choice experiments using laboratory populations collected from the wild in 2000, 2001 (spring and fall) and 2002 (spring). Experiments were performed in 2002 and 2003 in separate, independent, laboratories in Canada and Israel using two different mate choice designs: single choice experiments (in which females from one slope were allowed to choose between males from both slopes) and double choice experiments (in which males and females from both slopes were mixed in a single cage).

Methods

Experiments consisted of single and double choice trials conducted at Simon Fraser University in Burnaby, Canada and at the University of Haifa, in Haifa, Israel. Experiments were run in small and large plexiglass cages at Simon Fraser University and in glass bottles and large plexiglass cages at the University of Haifa. A total of 14 different experiments were conducted using populations from four different collections (Table 1).

Stocks and culture

Populations of *Drosophila melanogaster* were collected in 2000, 2001 spring (May) and fall (October), and 2002 spring (May) from opposing (north and south facing) slopes of the lower Nahal Oren, Mt. Carmel, Israel. Separate stocks of (year) 2000 flies at Simon Fraser University, Burnaby, were created from shipments of ≫100 flies that arrived in January and May of 2002 from Haifa and these were tested separately (see Table 1). 2001 spring and fall, and 2002 spring populations were all established in Burnaby in June of 2003 from shipments of ≫100 flies each. All stocks were created by combining bulk collections and isofemale lines, and were allowed to approach demographic equilibrium in the population cages for at least three months before mating trials were conducted.

Populations in Burnaby and Haifa were maintained in 4–8 plexiglass cages ($37 \times 27 \times 21$ cm^3) at populations sizes of >1000 flies per cage. Burnaby 2000 populations

Table 1 Summary of experimental set-up for mate choice tests.

Location	Population*	Choice test	Enclosure	Cross (f × m)†	Age‡
Both locations	2000§	Double	Large cages	20 + 20 × 40 + 40	6
	2000§	Single	Large cages	40 × 40 + 40	6
	2001 spring	Double	Small cages per bottles	10 + 10 × 10 + 10	4
	2001 fall	Single	large cages	40 × 40 + 40	5
	2002 spring	Single	Small cages per bottles	10 × 10 + 10 (Burnaby) and 13 × 13 + 13 (Haifa)	4
Haifa only	2001 spring	Double	Bottles	10 + 10 × 10 + 10	5
	2001 fall	Double	Bottles	10 + 10 × 10 + 10	5
Burnaby only	2000¶	Double	Small cages	10 + 10 × 10 + 10	4
	2001 fall	Double	Small cages	10 + 10 × 10 + 10	4

*Population: year and season flies were collected from the wild.
†Cross: numbers of females (south + north) × males (south + north) per replicate.
‡Age: number of days after enclosing.
§January shipment of flies.
¶June shipment of flies.

were initially maintained at 25 °C and 70% relative humidity on a 10 : 14 L : D cycle, but were changed to match conditions at Haifa in November of 2002: 25 °C, 55% relative humidity, 12 : 12 L : D cycle. All 2001 and 2002 populations in Burnaby were kept under the second treatment protocol exclusively.

Stock maintenance

Burnaby
Stocks were maintained using the following protocol. Once a week, a single bottle (Drosophila Products 8-oz. round bottom polypropylene container, product #AS-117; Applied Scientific, San Francisco, CA, USA) with 50 mL of standard growth medium (water, molasses, agar, cornmeal, yeast, propionic acid, penicillin and streptomycin) and fresh yeast (Fleischmann's Quick-Rise instant yeast, Burns Philip Food Ltd, La Salle, Canada) was placed in each population cage for egg laying. After approximately 200 eggs per bottle were laid, bottles were removed, capped and left undisturbed for 1 week. Two bottles of food with fresh yeast, along with the previous week's egg laying, were then placed in each cage for one week and discarded before the next round of egg laying. Under this regime, there is no larval crowding and each egg–adult generation was truncated at 14 days of age.

Haifa
Stocks were maintained using a similar protocol with one alteration: four half-pint milk bottles with 20 mL of medium in each (water, sugar, agar, cornmeal, raisin, yeast and a mixture of propionic and phosphoric acids) and fresh yeast (Fleischmann's Quick-Rise instant yeast, Burns Philip Food Ltd.) were introduced in population cages for 36 h for egg laying. Thereafter, bottles were removed, capped and left untouched for a week. Another series of bottles with fresh medium, together with the previous week's egg laying, were placed in population cages for a week and discarded prior to the next egg laying cycle. This means that flies in Israel were cultured under higher larval densities than in Vancouver, but the egg-adult generation time was also truncated at two weeks.

Collection for mating trials

Burnaby
Eggs were collected at low density (approximately 200 eggs per bottle) from each slope. Bottles were cleared eight to eleven days post-laying, just before dawn, and virgins were collected approximately 6 h later. To avoid bottle effects, the virgin flies from all bottles of a single slope were mixed in cages and then aspirated out in small aliquots (~50 flies) for sexing. Flies were sexed under light CO_2 and stored in food bottles. Holding densities in the initial 2000 population experiments varied by sex with 60 flies per bottle for females and 75 flies per bottle

for males. This was changed to 50 flies per bottle (regardless of sex) for all subsequent experiments. Dried yeast was added to female bottles only.

Mating trials were conducted when flies were 4–6 days post-eclosion. One day prior to mating trials, all flies were released into cages and then aspirated into yeasted vials. In the initial 2000 population experiments vial densities of 40 males and 20 females were used, but this was changed to 10 flies per vial (regardless of sex) for all subsequent experiments. To permit their identification, males (for use in the single choice experiments), and both males and females (for use in the double choice experiments), were marked by adding food colouring (red or blue; Food-Club brand, Scott-Bathgate Ltd, Winnipeg, Canada) to the live yeast in the holding vials as described in Mooers *et al.* (1999). Populations were reciprocally marked to balance any colour effects. As in our past studies (Rundle *et al.*, 1998; Mooers *et al.*, 1999; Rundle, 2003), no such effects were seen; of the 5006 matings observed in Burnaby, 50.6% involved red males, which does not differ from random expectation (χ^2-test with p-hat = 0.5, $\chi_1^2 = 0.63$, $P = 0.43$). None of the individual experiments differ from random expectation as well ($\chi_1^2 < 3.43$, $P > 0.064$ in all cases).

Haifa
An analogous protocol was used to collect the virgin flies for mating trials, except flies were collected from bottles with approximately 500 eggs laid. Holding densities in testing populations were 50 flies per half-pint bottle (regardless of sex). Fresh yeast was added only to female bottles. One day prior to a mating experiment, flies of a given sex were mixed (to decrease possible bottle effects), counted, and introduced into yeasted vials under light CO_2 anesthesia (10 flies per vial for the experiments in large plexiglass cages and 13 flies per vial for single female choice experiments in half-pint bottles). Males and females (double choice experiments) and males only (single choice experiments) were marked by adding green food coloring (Food-Club brand, Scott-Bathgate Ltd) to the live yeast in the holding vials. Marking was alternated between trials to balance any effects. However, no such marking effects were observed; of the 1226 matings observed, 49.02% involved marked males (χ^2-test with p-hat = 0.5, $\chi_1^2 = 0.47$, $P = 0.49$). Marking effects were also absent from individual experiments ($\chi_1^2 < 1.77$, $P > 0.18$ in all cases).

Mating trials

Mating trials were run in one of three types of enclosures: large plexiglass cages ($37 \times 27 \times 21$ cm^3) covered on all exterior sides except the top with white paper (to avoid any visual disturbance of the flies), small plexiglass cages ($12 \times 12.5 \times 12$ cm^3), also covered on all exterior sides except the top with white paper, and half-pint glass milk bottles.

Burnaby

Flies were released sequentially (males first) and mating pairs were aspirated out every 5–12 min (depending on mating rates) until 60% of the possible matings were obtained or 1 h had elapsed. Mating trials were conducted under ambient conditions: a mean temperature of 24.5 °C (range: 23–26 °C) and a mean relative humidity of 41.5% (range: 35–50%), under 'full-spectrum' fluorescent lights (Sylvania Octron CRI = 85, 3500 K).

Haifa

Flies were released sequentially (males first) into the mating chambers or bottles. Mated pairs were aspirated out every 3 min until ∼60% of the total possible number of matings had been obtained. Mating trials were conducted between 9:30 a.m. and 12:30 p.m. at 23 (±1 °C) and ∼40–50% relative humidity, under fluorescent lights (Leuci Gemini white, 4000 K).

Data analysis

Individual matings within an enclosure are not independent of one another because the relative frequencies of the different types of flies change as the trial proceeds and flies are mated, and because cage effects are shared among individuals. This precludes many forms of analyses that rely on independent choice by both males and females (see, e.g. Casares *et al.*, 1998; Naisbit *et al.*, 2001). Our analyses therefore used separate enclosures as replicates and were conducted in two main parts. In the first, the significance of nonrandom mating was evaluated for each experiment by calculating an index of assortative mating for every enclosure. The mean index

for all replicate enclosures within an experiment was then compared to the expected mean under random mating. One-tailed t-tests were used when data met assumptions of normality (Shapiro–Wilk *W*-test with $\alpha = 0.05$); cases that violated this assumption were tested using the Wilcoxon signed ranks test (as noted in Table 2).

For single choice tests, the proportion of homotypic matings (arcsin square root transformed) was used as the index of assortative mating for each cage. For double choice tests, the assortative mating index *Y* was calculated (Bishop *et al.*, 1975) for each cage. This index varies from −1 (perfect negative assortative mating) to +1 (perfect assortative mating) with 0 indicating random mating (for details see Rundle *et al.*, 1998). *Y* cannot be calculated if all possible mating types are not represented in a cage. This occurred in three data sets (Burnaby populations: 2000 (four cages), 2001 spring (four cages) and 2001 fall (one cage)). In these cases, one was added to all mating types collected from the cage, permitting *Y* to be calculated.

In double choice trials, we also measured female mate preferences separately by slope. To do this, the proportion of homotypic pairs formed by females from each slope was calculated in the same way as single choice data. While this analysis provides separate information about the mating preferences of the two types of females (which can be useful for interpreting significant assortative mating), it is not ideal because the proportion of homotypic matings for the two types of females (North facing slope or NFS and South facing slope or SFS) from a single cage are not independent. We thus refrain from statistical tests of these data.

Table 2 Results from parallel Israeli *D. melanogaster* mate choice experiments conducted in Canada and Israel.

| Trial | Population | Age | Location | n (cages)‡ | Mean % homotypic pairs§ *P* (one-tailed)¶ | | Mean *Y* value§ *P* (one-tailed)¶ |
					SFS female	NFS female	
Single choice	2000	4	Haifa*	8	0.62 [0.57, 0.68] 0.00020	0.56 [0.50, 0.62] 0.016	–
		6	Burnaby*	8	0.62 [0.57, 0.68] (0.012)	0.50 [0.41, 0.59] 0.48	–
	2001 (fall)	5	Haifa*	14	0.54 [0.46, 0.62] 0.10	0.61 [0.50, 0.72] 0.0008	–
		4	Burnaby*	36	0.49 [0.42, 0.54] 0.80	0.58 [0.54, 0.65] (0.0001)	–
	2002 (spring)	5	Haifa†	24	0.63 [0.52, 0.71] (0.0001)	0.59 [0.45, 0.71] (0.004)	–
		4	Burnaby†	30 (SFS)/29 (NFS)	0.62 [0.50, 0.80] (0.001)	0.44 [0.40, 0.57] (0.98)	–
Double choice	2000	4	Haifa*	16	0.56	0.58	0.14 [0.069, 0.22] 0.0001
		6	Burnaby*	16	0.47	0.46	−0.058 [-0.23, 0.025] 0.86
		4	Burnaby†	64	0.52	0.51	0.015 [-0.17, 0.22] (0.20)
	2001 (spring)	4	Haifa†	12	0.44	0.42	−0.14 [-0.37, 0.09] 0.96
		4	Burnaby†	59	0.46	0.58	0.0072 [-0.17, 0.27] (0.34)
		5	Haifa†	12	0.58	0.66	0.25 [0.13, 0.38] 0.0001
	2001 (fall)	4	Burnaby†	28	0.40	0.54	−0.085 [-0.31, 0.14] 0.92
		5	Haifa†	12	0.64	0.65	0.30 [0.18, 0.45] 0.0001

*Trials conducted in large plexiglass cages.
†Trials conducted in small plexiglass cages or glass bottles.
‡Number of trials per slope for single choice experiments, total number of trials for double choice experiments.
§50% quantile range about median.
¶*P*-values in parentheses are from Wilcoxon signed-rank tests, others from 1-sample *t*-tests.

The second part of the analyses combined data from multiple experiments to permit quantitative tests of the effects on assortative mating of our design variables (e.g. location of experiments, collection date, and slope of origin). These analyses used a general linear model framework that again treated enclosures as replicates (conducted using JMP Version 3.1.5). Because single and double-choice tests require different indices of assortative mating, data from these experiments could not be combined. Separate analyses were therefore performed on the combined data from all single and double-choice trials. In the analysis of the single choice trial data, we tested the affects on assortative mating (natural log-transformed) of collection date, laboratory, female population, and their interactions. We started with the full model and used backward elimination to remove non-significant interaction terms to arrive at the minimal adequate model describing the data. Using data from all of the double choice trials, we tested the affects on assortative mating of collection date, laboratory, and their interaction. Because only two factors were considered, backward elimination was not necessary.

Results

Single choice trials

Four of six trials using SFS females produced a significant proportion of homotypic mating: two in Burnaby and two in Haifa, involving the 2000 and 2002 spring collections (Table 2). In contrast, SFS females from 2001 fall mated (or were mated) randomly both in Burnaby and in Haifa. NFS females also exhibited a significant excess of homotypic mating in four of six trials: 2000 and 2002 spring populations tested in Haifa, and 2001 fall collections in both Haifa and Burnaby (Table 2).

Examining the combined data from all single choice trials, backwards elimination in the GLM produced a significant model with four terms ($r^2 = 0.14$, $F_{6,227} = 6.322$, $P < 0.0001$). Collection (partial $F_{2,227} = 7.86$, $P < 0.001$) and the effect of laboratory (partial $F_{1,227} = 7.44$, $P < 0.01$) are significant predictors of strength of assortative mating, and slope is marginally significant on its own (partial $F_{1,227} = 3.73$, $P = 0.06$). The only significant interaction was collection × slope (partial $F_{2,227} = 13.92$, $P < 0.0001$), which indicates that the difference in female preference between slope lineages varied among collection dates.

Double choice trials

Three of four experiments showed significant positive assortative mating in Haifa: 2000, 2001 fall, and 2001 spring collections (Table 2). In contrast, none of the four experiments conducted in Burnaby showed significant positive assortative mating (Table 2).

The GLM of double-choice trials produced a poorly fit model with three terms ($r^2 = 0.09$, $F_{5,203} = 3.87$, $P < 0.01$). Neither of the main effects were nominally significant (collection: partial $F_{2,203} = 2.05$, n.s.; laboratory: partial $F_{1,203} = 2.87$, $P = 0.09$) but their interaction was (partial $F_{2,203} = 5.88$, $P < 0.01$). This significant laboratory × collection interaction indicates that differences in the strength of assortative mating measured in the two laboratories varied by collection date.

Discussion

Initial studies of *D. melanogaster* from 'Evolution Canyon' suggested that this pair of populations represented an example of reproductive isolation due to adaptive divergence arising in the face of gene flow. Evidence for such adaptive divergence between the populations (Nevo *et al.*, 1998; Rashkovetsky *et al.*, 2000), substantial genetic differentiation (Michalak *et al.*, 2001), behavioural and life-history differences (Iliadi *et al.*, 2001), and highly significant same-slope mate preference exhibited by both females and males in single and double choice tests (Korol *et al.*, 2000), were consistent with the early stages of parapatric ecological speciation (Schluter, 2000). The results were also consistent with reports for other members of the north- and south-facing slope communities: decreased fertility of between slope crosses of *Sordaria fimicola* (Nevo, 2001) and nonrandom mating in *Acomys cahirinus* (E. Nevo, pers. comm.) and *Drosophila simulans* (S. R. Singh, E. Rashkovetsky, K. Iliadi, E. Nevo & A. Korol, unpublished).

Recent studies, however, have called this hypothesis into question. These studies failed to find any significant genetic differentiation across microsatellite (Colson, 2002, Schlötterer & Agis, 2002) or *Acp* loci (Panhuis *et al.*, 2003) between flies from opposing slopes and provided no evidence of significant population sub-structuring within the canyon (Colson, 2002; Schlötterer & Agis, 2002; Panhuis *et al.*, 2003). Additionally, recent double mate choice trials using older collections (1995) and older (6–10-day old) flies produced no evidence of sexual isolation (Panhuis *et al.*, 2003).

Consistent with these more recent findings, our present study does not offer compelling support for the strong and consistent reproductive isolation initially found between these populations, at least when tested outside of Israel. Although non-random mating was observed in the majority of both single and double choice experiments conducted in Haifa (and omnibus tests across all the reported experiments would support significant assortative mating), results were different in Burnaby and appeared to depend on the type of mating trial conducted. In single choice trials, preferences for same slope partners varied among collection dates. More notably, in double choice trials we failed to detect significant assortative mating in any of four separate experiments involving 167 replicate mating cages and almost 8000 flies. These

trials were conducted over several months by different sets of experimenters and used populations from all three collection dates. That nonrandom mating was weaker in Burnaby than in Haifa is confirmed by the significant laboratory effect for single choice trials and the laboratory × collection interaction for double choice trials shown in the GLM analyses of the combined data sets.

Why preferences for same slope partners was consistently weaker in all tests conducted in Burnaby is not known, although it is possible that these populations were affected in some way by shipment or, in the 2000 populations, by the initial higher humidity at which they were kept. Subtle differences in experimental protocol may also have had an effect. One potentially significant difference involved the rearing density of larvae, where stocks in Israel came from bottles with 36 h of egg-laying, rather than 1–2 for those in Burnaby. The lack of any strong assortative mating in the small cage experiments may also be ascribed to differences in handling, which have been shown to have subtle but strong effects on mate choice in *D. melanogaster* (see, e.g. Dukas & Mooers, 2003). Finally, there seems to be a pattern associated with fly age: trials using 5-day-old flies tended to give higher proportions of homotypic matings than did those using four or 6-day-old flies (Table 2; compare especially 2001 spring double-choice trials in Haifa). Unfortunately, because of multicollinearity we are unable to test the effects of age (or handling) directly using our current data (neither were part of our original design). We note that Panhuis *et al.* (2003) used 6–10-day-old flies in their trials and suggest that more work be conducted looking at this phenomenon.

Nevertheless, it is important that significant preferences for same slope partners were detected in three of the six single choice experiments run in that lab. In addition, trends in these six single choice experiments were similar between labs (proportion homotypic mating, $r = 0.75$, $P = 0.08$, $n = 6$). Consistent differences in male vigour between slopes cannot explain our single choice results because both slopes exhibit significant homotypic mating in both labs. Only the results from the 2001 fall collection are consistent with lowered male vigour for SFS males: more NFS males than SFS males mated in the 2001 fall double choice experiments in Burnaby ($\chi_1^2 = 5.14$, $P = 0.02$); in Haifa NFS females constituted 59.6% of the mated females in the parallel experiment ($\chi_1^2 = 5.57$, $P = 0.02$); and finally in both labs, single choice experiments showed significant assortative mating only for NFS females (Table 2). However, if male vigour were the only behavioural difference between slopes we would expect consistent assortative mating across collections for that slope alone and dissassortative mating in trials involving females from the opposing slope. We do not find this. Taking all the collections together, the results from the two labs are consistent with behavioural differences between the slopes that lead to assortative mating under some circumstances.

Finally, in neither ours, nor Korol *et al.*'s original experiments (2000), did homotypic matings exceed 64.2% of total matings. At face value, this seems a fairly weak behavioral barrier to gene flow and it is conceivable that premating isolation could be stronger in nature. Isolation in the lab could weaken as a by-product of adaptation to laboratory conditions, or because handling and testing under laboratory conditions removes important environmental cues used in mating (e.g. known differences in oviposition temperature and other slope-specific differences observed between the populations; Nevo *et al.*, 1998; Rashkovetsky *et al.*, 2000). If behavioural isolation is stronger in nature, the absence of significant genome-wide genetic differentiation between the populations can best be reconciled with the behaviourally mediated speciation model if the population split began very recently (Wu, 2001). If this is true, there should still be a small number of microsatellite loci, tightly linked to selected chromosomal regions, that differ between the populations (Schlötterer & Agis, 2002). Consistent (across two collections) differences have been found in one genomic region (DM18774) between the slopes (Colson, 2002). Indeed, in parapatric models of divergence with gene flow, divergent adaptive trait complexes themselves may be better evidence for divergent selection than genetic distances estimated using molecular markers (see S. R. Singh, E. Rashkovetsky, K. Iliadi, E. Nevo & A. Korol, unpublished). Alternatively, if the level of assortative mating seen in the lab is representative of the field, then little genetic divergence may be expected except at specific loci under strong disruptive selection. Incipient speciation status of these populations would then seem premature, regardless of the evidence that the communities in which the flies find themselves seem quite divergent (see Nevo, 1995).

In conclusion, and together with other recent findings (Colson, 2002; Schlötterer & Agis, 2002; Panhuis *et al.*, 2003), our study suggests that more detailed work is necessary on this potentially important system before ascribing biological significance to the nonrandom mating that has been reported to date. Further research on within- and between-population mating behaviour, on hybrid fitness, and on viability of flies under slope-specific conditions would be useful in determining in what ways these populations are diverging.

Acknowledgments

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