

Evidence for male-biased dispersal in Lake Malawi cichlids from microsatellites

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Abstract

This study addressed within-population dispersal patterns among the species-rich Lake Malawi cichlids, specifically among the rock-dwelling mbuna group. Relatedness values were calculated for 160 individuals belonging to two species from known locations in the field by screening six highly polymorphic microsatellite loci. The results from both species indicate that spatially adjacent females have higher average relatedness values than those separated by larger distances, but that this pattern is reversed in males. This therefore provides firm evidence for male-biased dispersal within the Malawian cichlid flock.

Keywords: cichlids, Lake Malawi, male-biased dispersal, microsatellites, relatedness

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Introduction

The East African Great Lake cichlid flocks have long been of interest due to their remarkable diversity and rapidity of speciation. Despite this, many aspects of their biology remain poorly understood. The current study focused on the *Pseudotropheus* (*Maylandia*) complex that belongs to the mbuna, a group of rock-dwelling cichlids from Lake Malawi. (Stauffer *et al.* (1997) recently proposed *Metriaclima* as a new genus name for this complex.) Our knowledge of dispersal patterns within the mbuna group and specifically within the *Pseudotropheus* (*Maylandia*) complex is extremely limited. There is strong empirical evidence indicating very low (if any) dispersal between populations occupying adjacent (~ 500 m distant) rocky headlands (van Oppen *et al.* 1997a; Arnegard *et al.* 1999). This indicates that these species are philopatric and do not disperse over areas of deep water and/or sandy stretches of shoreline. Nothing, however, is known about within-population dispersal patterns, which leaves us with little understanding of population dynamics. Furthermore, many studies have suggested that sexual selection may have played an important role in the speciation of the cichlid flocks of Lakes Malawi and Victoria (e.g. Dominey 1984; Turner & Burrows 1995; Seehausen *et al.* 1997; van Doorn *et al.* 1998), and sex differences in dispersal patterns may profoundly

influence the feasibility of this process leading to diversification of populations that are not completely geographically isolated. In strongly sexually selected species, males are expected to be much less discriminating in mating than females (Andersson 1994). Assuming populations have diverged in both male courtship traits and corresponding female preferences, males migrating between demes will probably be willing to mate with resident females, but these females, preferring local males, will reject them. In contrast, migrant females will prefer to mate with males of their natal deme, but such males will be absent or rare in the local environment, so they will probably accept resident males (assuming preference is not absolute, which has been demonstrated by the production of hybrids in the laboratory; M. E. Knight and G. F. Turner, unpublished; Cragon de Caprona 1986), which would lead to introgression. Thus, female-biased dispersal (females moving further from their natal areas than males) will probably have a far stronger inhibitory effect on speciation in sexually selected species than male-biased dispersal.

This study centred on two members of the *Pseudotropheus* (*Maylandia*) complex: *P. zebra* and *P. callainos*, which occur sympatrically in high densities (approximately 0.6/m² and 0.4/m², respectively; M. E. Knight, unpublished) at Nkhata Bay, Malawi. Within interbreeding populations (Knight *et al.* 1998; van Oppen *et al.* 1998), rare female morphs (such as *P. callainos* 'blotched', see Konings (1995)) have often been observed occurring in clusters in the field:

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several are seen within a very small area but none in others. This patchy distribution suggests that either 'like' female morphs are grouping together actively or that individuals do not move far from their natal site, remaining in the same locality for life. The latter inference is supported by studies reporting accurate homing abilities and site fidelity in mbuna species (Hert 1992) along with observational data on female ranging patterns in the field (M. E. Knight, unpublished).

If individuals really do not move from their natal sites, however, how is inbreeding avoided? Studies using microsatellite markers have provided no evidence for inbreeding in the *Pseudotropheus* populations discussed here (van Oppen *et al.* 1997a). We are therefore faced with the conflict of apparently no dispersal but no indication of inbreeding. This conundrum could be explained by male-biased dispersal: females staying within the natal area but males dispersing throughout the local population. Such a hypothesis would account for the absence of inbreeding as well as for the apparently clumped distribution of rare female morphs. An equally credible hypothesis, however, would be that neither sex actively disperses but females ready to spawn travel away from their natal area to find a mate and/or travel from their home range to release their mature fry (females brood eggs in their mouths until the fry are fully developed). Little is known about the behaviour of females but if homing abilities are as developed as evidence suggests, such behaviour would be entirely feasible.

Conventional studies investigating dispersal among organisms demand long-term observations of known individuals. Because we currently do not know the age at which individuals may disperse, any such study would have to include fry. This would involve following females both before and after spawning as well as tracking the released fry. Fry are extremely difficult to catch, spending the majority of time under rocks and crevices, and are additionally notoriously difficult to positively identify. Gaining comprehensive data on dispersal among these species using an observational technique would therefore be at best extremely problematic. Recent advances in molecular techniques coupled with their increasing accessibility have, however, opened up new opportunities for tackling such problems. If individuals really do not disperse far from their natal sites, we would expect individuals living in close proximity to be more closely related, on average, than individuals taken at random from the population.

The rationale of this present study was to sample individuals from known locations on a small spatial scale (mapping coordinates for each individual to allow for pairwise distances to be calculated), and obtain estimates of relatedness (Grafen 1985; Queller & Goodnight 1988; Queller *et al.* 1993) by screening microsatellite loci. With

these data it would then be possible to assess whether there is a relationship between relatedness and geographical distance for each sex and further, whether this relationship is the same in both sexes, thereby assessing whether dispersal is likely to be occurring and if so, whether it is sex-biased.

Relatedness values calculated from microsatellite loci have been the basis of several other studies examining parentage and population structure: in plants (Dow & Ashley 1996), placental mammals (Blouin *et al.* 1996; Garza *et al.* 1997; Smith *et al.* 1997), marsupials (Moritz *et al.* 1997; Taylor *et al.* 1997), aphids (Oldroyd *et al.* 1997), social insects (Bourke *et al.* 1997; Chapuisat *et al.* 1997; Chapuisat 1998; Strassmann *et al.* 1998) as well as in salmonids (Hansen *et al.* 1997; Fontaine & Dodson 1999). This study built on such applications by directly correlating relatedness with distance on a small spatial scale to gain an understanding of hitherto unknown dispersal patterns without relying on paternity exclusion. Paternity exclusion was considered impractical for the present study. First, the high density of fish meant that it was not feasible to sample all individuals in an area. Furthermore, the high incidence of multiple paternity (Kellogg *et al.* 1995; Parker & Kornfield 1996) meant that most fry in a clutch would be half-sibs, which are much more difficult to distinguish from unrelated individuals.

Materials and methods

Sample collection

Two 20 m Nylon string lines were laid at right angles to each other on the substrate at a site on Nkukute Point, Nkhata Bay, Malawi, one running south to north, the other east to west, at a depth of 2.8–4.7 m (see Fig. 1).

Fish were caught individually from the transect space using a 10 × 2 m monofilament 'curtain' net and SCUBA equipment. A total of 160 individuals were caught: 'females' (see below) and territorial males of both *Pseudotropheus callainos* and *P. zebra*. Catching was concentrated in particular areas of the transect (areas 1–8, Fig. 1). The exact location of each fish prior to capture was marked with a numbered ceramic tile. These tiles were subsequently mapped relative to the transect lines using a tape measure, resulting in a known location for each individual. Fin clips from the left pectoral fin were taken and preserved in individual vials filled with NaCl–dimethylsulphoxide (DMSO) buffer (Seutin *et al.* 1991) for microsatellite analysis. The entire fish was then preserved in formalin and transported back to the UK for subsequent morphometric analysis and sexing. Fish were transferred to an alcohol preservative upon arrival in the UK. It was necessary to determine sex by dissection as females and immature males cannot be reliably distinguished by other means.

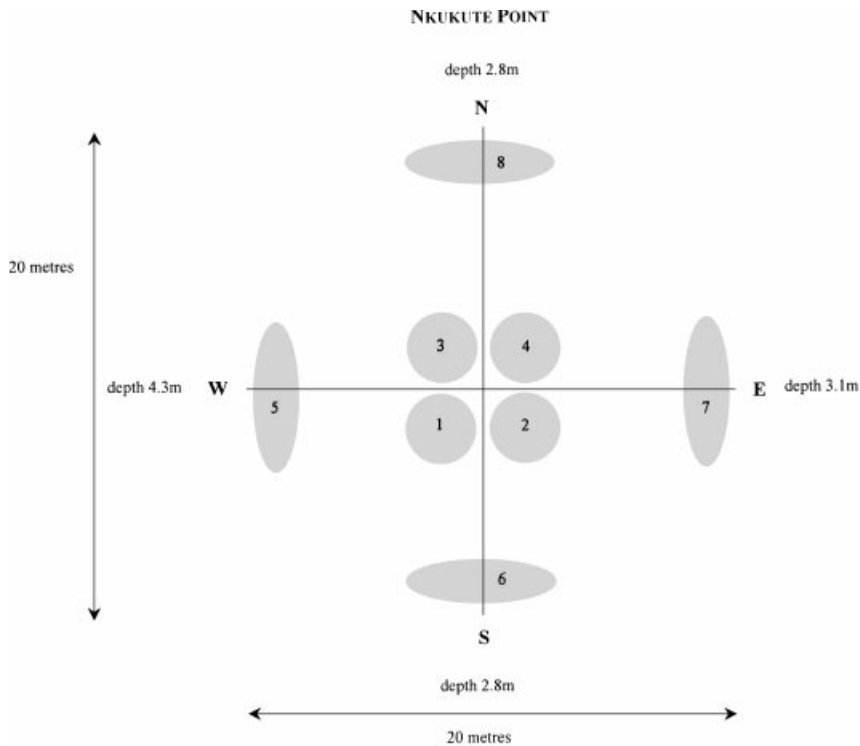


Fig. 1 Transect position at Nkukute Point, Nkhata bay, Malawi, showing the eight areas where catching effort was concentrated.

Microsatellite analysis

DNA was extracted following a chloroform-based protocol (Rico *et al.* 1992). Each individual was screened at six polymorphic microsatellite loci (the number of alleles within the population at each locus is shown in brackets for *P. zebra* and *P. callainos*, respectively): Pzeb1 [47,45], Pzeb2 [29,23], Pzeb3 [12,11] (van Oppen *et al.* 1997b), UNH002 [34,29] (Kellogg *et al.* 1995) [all perfect dinucleotide repeats]; Pzeb5 [6,2] (van Oppen *et al.* 1997a) [imperfect dinucleotide repeat]; Pzeb4 [10,4] (van Oppen *et al.* 1997a) [compound repeat]. Amplified samples were run on an ALF sequencer (Pharmacia) with internal standards as described in van Oppen *et al.* (1997a).

Calculating relatedness

A statistical estimate of the coefficient of relatedness (r) (originally formulated by Wright (1922), but often referred to as 'Hamilton's r ' (Hamilton 1964), see also, for example Maynard Smith (1998)) was calculated using the Macintosh computer program KINSHIP 1.2 (Goodnight *et al.* 1998). This pairwise relatedness coefficient (R) (Grafen 1985) is calculated between any two individuals by comparing the shared alleles of these individuals with the allele frequencies in the population as a whole, i.e. estimating whether any shared allele is present by descent or by chance given its frequency within the population (see Queller & Goodnight (1988)). This assumes that the average relatedness within

the population as a whole is 0 and R therefore varies between -1 and 1 (as opposed to the more familiar $0-1$ range of Hamilton's r).

All possible pairwise combinations were calculated by comparisons with allele frequencies already established for the overall Nkukute Point population (van Oppen *et al.* 1997a). In 15 individuals not all six microsatellite loci amplified. Because in these cases the estimate of R would be based on fewer loci, these individuals were excluded. Values for the relatedness coefficient (R) were calculated for each sex and species separately.

Distance calculation

All possible pairwise distances were calculated for each individual using a program written in Microsoft VISUAL C++, which calculated the Euclidean distances between pairs using the coordinates provided for each individual.

Statistical analysis

Values for relatedness (R) and pairwise distance (D) were sorted for each possible combination of individuals within each data subset. Data could not, however, be statistically analysed in this form, as although the sample size (number of individuals) in the species and sex categories ranged from 23 to 51, the number of pairwise comparisons within any one of these data sets far exceeded this. For example, for *P. zebra* males the number of individuals $n[\text{individ.}] = 23$

but the number of pairwise comparisons would be 253. In this form the number of degrees of freedom in any statistical test would be inflated, presenting a problem of pseudoreplication. Subsequent analyses therefore followed two different approaches, both based on n [individ.].

Comparison of individual regressions of R on D. For this analysis, a separate regression of R on D was calculated for each individual. The data set comprised all of the pairwise R - and corresponding D -values involving a particular individual. These R - and D -values were calculated only between individuals of the same sex and species. A regression of R on D was then calculated for each individual's values. This resulted in n [individ.] regressions for each sex of each species. The slope values (b) of all these individual regression lines were then statistically compared between the sexes within both species. High negative slope values indicate that an individual's close relatives are more likely to be found nearby. As some data were not normally distributed a nonparametric Mann–Whitney U -test was used to test the null hypothesis that there was no difference between the sexes in the slope values of individual regressions of R on D .

Comparison of means of ranked R-values. The second approach was based on data points that were the means of equivalent ranked R - (and corresponding D -) values for all individuals of a particular sex and species. For each individual the R -values were ranked in descending order. The first data point was thus calculated as the mean across all individuals' highest R -values and corresponding D -values. The second data point was the mean of all individuals' second highest R -value, etc. This gave $n - 1$ data points (where n = the number of individuals originally sampled). This is because an individual cannot have an R -value with itself. For each species the slopes of R on D were then compared between the sexes using Student's t -test (Zar 1996).

Results

Comparison of individual regressions of R on D

In both species, the slopes of the individual regression lines differed statistically between males and females (Tables 1 and 2). Females tended to have lower regression coefficients than males (Figs 2 and 3).

Comparison of means of ranked R-values

Comparing the slopes of R on D between the two sexes within each species (Zar 1996), there was a significant difference between the slopes in both *Pseudotropheus zebra* and *P. callainos* (*P. zebra*: $t = 2.03$, $P = 0.049$, d.f. = 44; *P. callainos*: $t = 2.90$, $P = 0.005$, d.f. = 79). In both species, female

Table 1 Mann–Whitney U statistic, comparing the slopes of the regression lines of R on D for individual male and female *Pseudotropheus zebra*

Sex	Cases (n)	Mean rank	Sum of ranks	U	P
Male	23	30.65	705.0	192.0	0.0208
Female	27	21.11	570.0		

Table 2 Mann–Whitney U statistic, comparing the slopes of regression lines of R on D for individual male and female *Pseudotropheus callainos*

Sex	Cases (n)	Mean rank	Sum of ranks	U	P
Male	51	48.56	2477	532.51	0.0046
Female	34	33.14	1094		

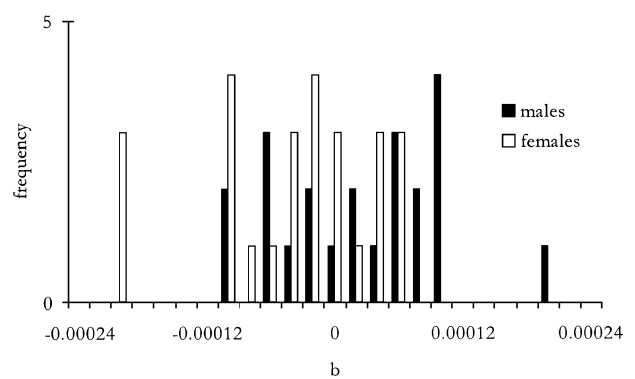


Fig. 2 Frequency distributions of the regression slope values (b) of relatedness on distance between male and female *Pseudotropheus zebra* (where for males $n = 23$, median value = 2.0×10^{-5} ; for females $n = 27$, median value = -2.0×10^{-5}). The regression slope values were calculated from each individual's pairwise relatedness and distance values with all other individuals of the same sex (see Materials and methods). Negative slope values indicate a negative relationship between relatedness and geographical distance.

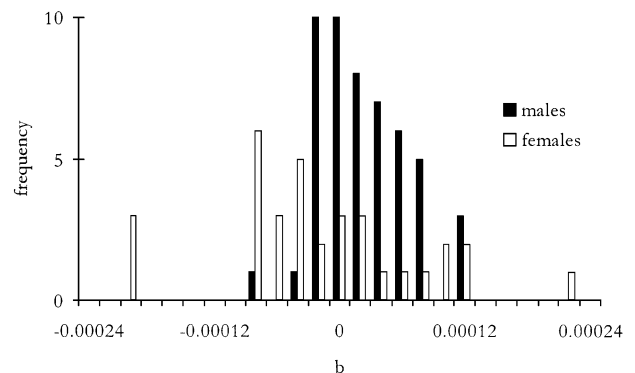


Fig. 3 Frequency distributions of the regression slope values (b) of relatedness on distance between male and female *Pseudotropheus callainos* (where for males $n = 51$, median value = 4.0×10^{-6} ; for females $n = 34$, median value = -5.0×10^{-5}). Regression slope values were calculated independently for each individual as detailed for Fig. 2.

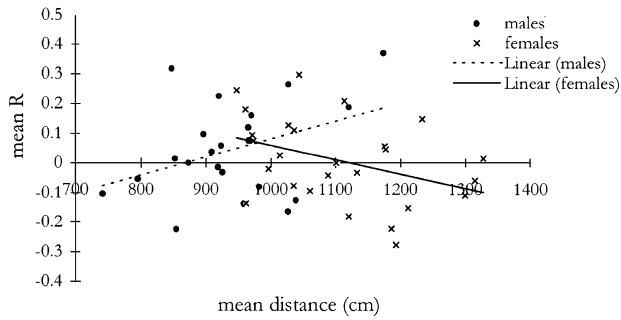


Fig. 4 Relationship between the means of ranked relatedness and corresponding distance values calculated separately for each sex for all *Pseudotropheus zebra* individuals (see Materials and methods). Relatedness shows a positive trend with distance in males (r [correlation coefficient] = 0.377, P = 0.083), and a negative relationship in females (r = -0.379, P = 0.056), although these trends are not statistically significant.

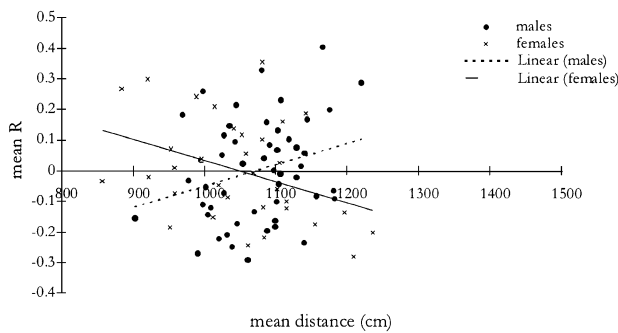


Fig. 5 Relationship between the means of ranked relatedness and corresponding distance values calculated separately for each sex for all *Pseudotropheus callainos* individuals (see Materials and methods). Relatedness shows a positive but nonsignificant trend with distance in males (r [correlation coefficient] = 0.274, P = 0.054), and a statistically significant negative relationship with distance in females (r = -0.373, P = 0.032).

pairwise relatedness values showed a negative relationship with distance (Figs 4 and 5; correlation coefficients (r) are listed in the figure captions), although this was found to be significantly different from zero only in *P. callainos* females. For males of both species this pattern reversed, with a positive trend found between relatedness and distance. However, in neither case was this relationship statistically significant.

Thus, both analyses indicate that females of both species are more likely to be found in proximity to close relatives than are males. This strongly suggests male-biased dispersal among these species.

Discussion

Many previous studies have determined small-scale dispersal patterns within populations by using microsatellite

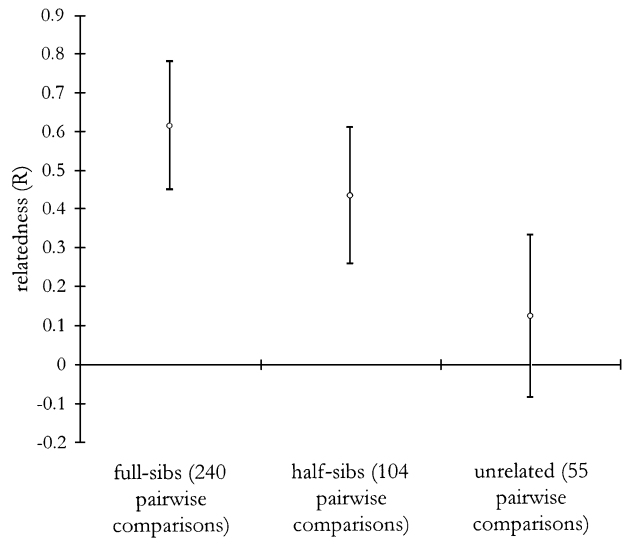


Fig. 6 Mean R -values (and standard deviation) found from pairwise comparisons of known relatives (from 45 individuals screened at six loci, data from Knight *et al.* (1998)). The values were calculated from a small number of broods (six). Three of these broods shared the same father and two independent mothers by chance shared several alleles. These factors have resulted in slightly elevated mean relatedness values than those expected (0.5, 0.25 and 0 for full-sibs, half-sibs and unrelated individuals, respectively).

genotypes to discern parentage using either direct or indirect (such as likelihood) methods (e.g. Hansen *et al.* 1997; Moritz *et al.* 1997; Taylor *et al.* 1997; Garza *et al.* 1997). Such an approach requires an accurate estimate of relatedness being available from the microsatellite (or other) loci being used, and/or a sampling procedure allowing (at least) the majority of individuals in the area to be sampled. For many reasons these may not always be available. In the case reported here, neither of these criteria were met. The estimate of relatedness calculated was known to be noisy from a previous laboratory study on individuals of known relatedness using the same six microsatellite loci as used here (Knight *et al.* 1998; Fig. 6). Second, population densities in these fish are extremely high in the field. Even within a very small area it would be extremely labour intensive, if not impossible, to catch and sample every individual of any one species. Further, because the only reliable method of sexing individuals other than territorial males of these species is by inspecting the gonads, such a sampling scheme would be unethical. Thus, although screening further loci will probably provide a better estimate of relatedness, this might not actually be useful. This is not only because all individuals have not been sampled, but also because even if individuals do not disperse from their natal area, we would still expect many individuals living in close proximity to be unrelated. No figures are available for mortality, but it is likely

that the majority of any one individual brood does not survive to adulthood. Furthermore, it is known that individual clutches laid by mbuna females are generally sired by many males (up to six: Kellogg *et al.* 1995; Parker & Kornfield 1996). Thus, within any single brood most individuals will be half-sibs, a relationship difficult to positively distinguish from that of unrelated individuals using relatedness estimates (e.g. Fontaine & Dodson 1999). The analytical techniques used here are robust to these problems, allowing an assessment of dispersal patterns based on general trends in relatedness values as opposed to accurate pairwise values, high values of which may simply not be found by chance in a subsample of a high-density population. This approach will probably be of use in many other studies.

Both analyses showed similar statistically significant trends: on a small spatial scale female relatedness values decline with distance whereas males show an opposite trend and are less likely to be related to individuals occupying adjacent space. This pattern was consistent in both *Pseudotropheus zebra* and *P. callainos*. Although the trend between relatedness and distance found in males was positive, the relationship was nonsignificant in both species and as such a biological interpretation would be premature. Should this result prove statistically robust in subsequent studies, however, male avoidance of proximity to close relatives would be one feasible explanation. Male-biased dispersal fits with observational evidence from the field, where rare female morphs are often found in clumped distributions, and resolves a paradox over the apparent lack of inbreeding. Only territorial males of these species are able, as far as is known, to spawn with females. Young males are unlikely to be able to command territories given that territory space appears to be a limited resource (M. E. Knight, unpublished). Thus, immature males will probably have to spend a length of time within the nonterritorial 'floating' population until they reach a size where they are able to compete with existing territorial males for space. Within this scenario, it is entirely feasible that immature males would not stay within their natal area (which females are here suggested to do), but range further afield. Unfortunately no data are as yet available on the acquisition of territories by males, nor do we have any information on the expected lifespans of individuals, although individual males have been observed to remain on the same territory for as long as 3 years (Robinson 1995).

These data showing relatedness values to be higher among spatially adjacent individual females would also suggest that females do not release their fry over a wide area but release them together, where the majority of surviving females may stay for life. Whether females travel to release their fry, however, cannot be inferred from these data.

Many studies have suggested that sexual selection may

have played an important role in the speciation of the cichlid flocks of Lakes Malawi and Victoria (e.g. Dominey 1984; Turner & Burrows 1995; Seehausen *et al.* 1997; van Doorn *et al.* 1998). Male-biased dispersal is more consistent with such a hypothesis than is female-biased dispersal, as it would be predicted in parapatric populations that the dispersal of females away from their natal deme would hinder the diversification of subpopulations in terms of male courtship traits and female preferences for those traits.

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