Mate recognition in a freshwater fish: geographical distance, genetic differentiation, and variation in female preference for local over foreign males

B. B. M. WONG, J. S. KEOGH & M. D. JENNIONS

School of Botany and Zoology, Australian National University, Canberra, Australia

Abstract

It often is assumed that more distant allopatry should reflect reduced rates of contemporary gene flow and/or greater divergence in mate recognition systems. This assumption, however, is rarely tested and may not always be appropriate. Here we investigated female preference for local and foreign males in a morphologically variable Australian freshwater fish, the Pacific blue-eye *Pseudomugil signifer*. Using a multidisciplinary approach that combined molecular phylogeography with conventional mate choice experiments, we found female blue-eyes spent more time in association with local males only when the alternative was a foreigner from a geographically and genetically more distant population. When offered the choice between two foreign males, females associated more with males from the population that was more closely adjacent to their own. Our results suggest that female preference for local over foreign males in blue-eyes may depend on how genetically and geographically separated populations are from one another.

Introduction

Reproductive isolation is a cornerstone of the speciation process. Mate recognition traits often vary spectacularly across the geographical range of a species (For examples, see Ptacek, 2000). Between populations, a range of stochastic and selective forces can operate to cause signals and receivers to diverge. For example, adaptation in response to local environmental conditions and/or sexual selection can lead to correlated evolution between signal and receiver as demonstrated, for example, in guppies *Poecilia reticulata* (Endler & Houde, 1995) and jumping spiders *Habroniattus pugillis* (Masta & Maddison, 2002). Isolation could also be mediated through genetic drift and founder effects (Tilley *et al.*, 1990; Tregenza et al., 2000). The resulting divergence of recognition traits has the potential to lead to premating reproductive isolation, where members of one sex no longer recognize foreign members of the opposite sex or strongly prefer locals.

Correspondence: Bob B. M. Wong, School of Botany and Zoology, Australian National University, Canberra, ACT, 0200, Australia.
Tel.: +612 6125 8057; fax: +612 6125 5573;
e-mail: bob.wong@anu.edu.au

Investigating patterns of divergence within species can therefore contribute to an understanding of the manner in which reproductive isolation might arise and how this isolation, in turn, may contribute to the process of speciation (Dagley *et al.*, 1994; Shaw, 2000; Mendelson & Shaw, 2002).

Female preference for local over foreign males has been reported in a range of taxa including snails (Rupp & Woolhouse, 1999), *Drosophila* (reviewed in Boake, 2002), fish (Endler & Houde, 1995), salamanders (Tilley *et al.*, 1990) and rodents (Pillay *et al.*, 1995; Pillay, 2000). Such results are often interpreted to mean that mating preferences have diverged in allopatry, resulting in assortative mate choice and premating reproductive isolation (e.g. Pillay, 2000). Females, however, do not always prefer local over foreign males. For instance, in guppies, a significant female preference for local males was found in some populations but not others (Endler & Houde, 1995; Magurran *et al.*, 1996; Houde & Hankes, 1997). Such findings underscore the need for caution in simply assuming that signals and preferences will diverge concomitantly. While considerable effort often is focussed on documenting geographical variation in male traits (e.g. Castellano *et al.*, 2000), greater attention

should be placed on actually testing variation in female preferences (Panhuis *et al.*, 2001). Such tests can be illuminating and are easily performed through mate choice experiments (Ptacek, 2000). For example, investigating preference for homotypes has been instrumental in revealing cases of evolutionary mismatch between male traits and female preferences (e.g. Ryan & Rand, 1990; Hill, 1994; Basolo, 1995; Simmons *et al.*, 2001) and also whether individuals preferentially mate with unrelated genotypes to avoid the costs of inbreeding (Rupp & Woolhouse, 1999). Both situations could facilitate interbreeding rather than premating reproductive isolation (Rupp & Woolhouse, 1999; Panhuis *et al.*, 2001).

Female preference for homotypes also could depend on how distantly separated populations are from one another (Tilley *et al.*, 1990; Endler & Houde, 1995). It is generally assumed that divergence of mate recognition systems should be correlated with the degree of genetic differentiation (e.g. Strecker & Kodric-Brown, 1999; de Kort & ten Cate, 2001). Few studies, however, explicitly test for such an association and, often, genetic differentiation is simply inferred from geographical distance (e.g. Simmons *et al.*, 2001). This is because more distant allopatry is often believed to reflect lower rates of contemporary gene flow, longer histories of isolation and more divergent selective regimes (Tilley *et al.*, 1990; Endler & Houde, 1995). Actual tests of this assumption are uncommon. A pioneering attempt was made by Tilley *et al.* (1990) in their study on salamanders, *Desmognathus ochrophaeus*. They investigated the evolution of reproductive isolation by relating patterns of assortative mating with levels of genetic divergence and geographical distance among salamander populations. Whereas the study successfully showed that sexual isolation increased with geographical distance, the link between distance and genetic divergence could not be demonstrated unequivocally with the use of allozyme data (Tilley *et al.*, 1990). An explicit test of assumptions underlying the effects of distant allopatry is important because the presumed link between geographical distance and genetic differentiation may not always occur. Tregenza *et al.* (2001), for example, recently found that reproductive isolation in European meadow grasshoppers *Chorthippus paralleus* had occurred rapidly because of founder effects, causing some populations to be more genetically divergent than geography alone might otherwise predict.

The relatively recent field of molecular phylogeography offers important possibilities for studies of speciation and mate recognition because it can provide the necessary map of phylogenetic relationships and genetic divergence between populations (Wiens *et al.*, 1999; Avise, 2000; Barraclough & Nee, 2001). These data are valuable because a sound understanding of the underlying phylogeographical relationships, coupled with morphometric and behavioural data relevant to mate choice decisions, help us understand the relative import-

ance of reduced gene flow in the divergence of mate recognition systems (Avise, 2000).

Determining the underlying genetic structure among populations used in experimental behavioural work is still rare, despite the now widespread acknowledgement among evolutionary biologists that phylogenetic history is important in the interpretation of comparative data (Blomberg & Garland, 2002). Here we sampled populations from throughout the range of a morphologically variable species of fish, the Pacific blue-eye *Pseudomugil signifer* Kner. We then used our molecular phylogenetic results to strategically pick sets of populations that were genetically divergent and genetically similar. These populations, in turn, were used in mate choice experiments to test the assumption that mate recognition systems should diverge with more distant allopatry as a consequence of reduced gene flow (Tilley *et al.*, 1990; Endler & Houde, 1995). We found that females spent more time in association with local males only when the alternative was a foreigner from a geographically and genetically more distant population.

Methods and results

Study species

The Pacific blue-eye *P. signifer* is a small, sexually dimorphic freshwater fish with a widespread distribution across eastern Australia (McGlashan & Hughes, 2002). Males are larger than females and possess spectacular fin extensions that are lowered and raised during both agonistic encounters and courtship (Wong & Jennions, 2003). Although the species is sexually dimorphic, it also exhibits substantial inter-population variation in male body size and fin length. The results of recent molecular work suggest extensive genetic divergence among populations at a broad geographical scale, as mitochondrial DNA sequence data identified northern and southern populations as representing two distinct genetic clades (McGlashan & Hughes, 2002; Wong *et al.*, 2004) The results of previous mate choice experiments suggest that Pacific blue-eyes are highly suitable for behavioural work and experimental protocols have already been established (Wong & Jennions, 2003; Wong, in press).

Molecular phylogeny

Pseudomugil signifer has an extensive distribution along the east coast of Australia from Eden in southern New South Wales to Cape York Peninsula in far north Queensland. We strategically sampled individuals from 10 populations across the species' range. The closely related *P. mellis* was used as an outgroup in our analyses giving us 11 samples in total. For each sample we targeted a 633 base pair (bp) DNA fragment of the ATP synthase six mitochondrial gene. The target fragment

was amplified using primers ATP8.2L and COIII.2 (McGlashan & Hughes, 2002). All laboratory procedures are as in Keogh *et al.* (2000). Parsimony, neighbour-joining and maximum likelihood analyses were performed on the data using PAUP* Version 4.0 b10 (Swofford, 2002). For our parsimony analyses we used unweighted data and also used the ti/tv ratio estimated from the data via maximum likelihood. A total of 1000 bootstrap pseudoreplicates were performed in the parsimony analysis to examine the relative support for each branch.

The edited alignment is 633 bp in length. Aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial genetic code. No premature stop codons were observed, so we conclude that all sequences obtained are true mitochondrial copies. For the entire data set, a total of 203 sites were variable and 127 parsimony informative. The actual ti/tv ratio estimate via maximum likelihood for the entire data set was 2.81. A single tree topology was recovered regardless of the type of phylogenetic analysis so in Fig. 1 we show the results for an unweighted parsimony analysis (length = 307 steps, CI = 0.79, RI = 0.82, RC = 0.65, HI = 0.21). The tree is fully resolved with high bootstrap support for all nodes. *Pseudomugil signifer* comprises two major clades, a southern clade with populations from southern New South Wales to central Queensland and a northern clade with populations from far north Queensland.

Based on our phylogeny we chose four focal populations for behavioural experiments comprising two pairs of geographically and genetically closely related populations, one pair from southern Australia and one pair from northern Australia (Fig. 1, bold). Lake Willinga (LW) and Nelligen Creek (NC) populations are part of a southern clade separated by a distance of approximately 50 km. They group together with 99% bootstrap support and differ by only a single base pair resulting in a Jukes–Cantor (Jukes & Cantor, 1969) genetic difference of only 0.16%. Stone River (SR) and Ross Creek (RC) populations are part of a northern clade separated by a distance of approximately 200 km. The two northern populations group together with 100% bootstrap support and differ by 8 bp, resulting in a genetic difference of 1.28%. Northern and southern populations differ genetically by 17.14–17.74%.

Morphometrics

We also quantified six male morphometric traits from our four focal populations to obtain a measure of morphological similarity between the populations: front dorsal fin length, second dorsal fin length, anal fin length, ventral fin length, pectoral fin length, and standard length. These traits were chosen because they previously have been reported to differ among populations and also because of their role in male courtship display (Hadfield *et al.*, 1979; Wong & Jennions, 2003). We did a clustering
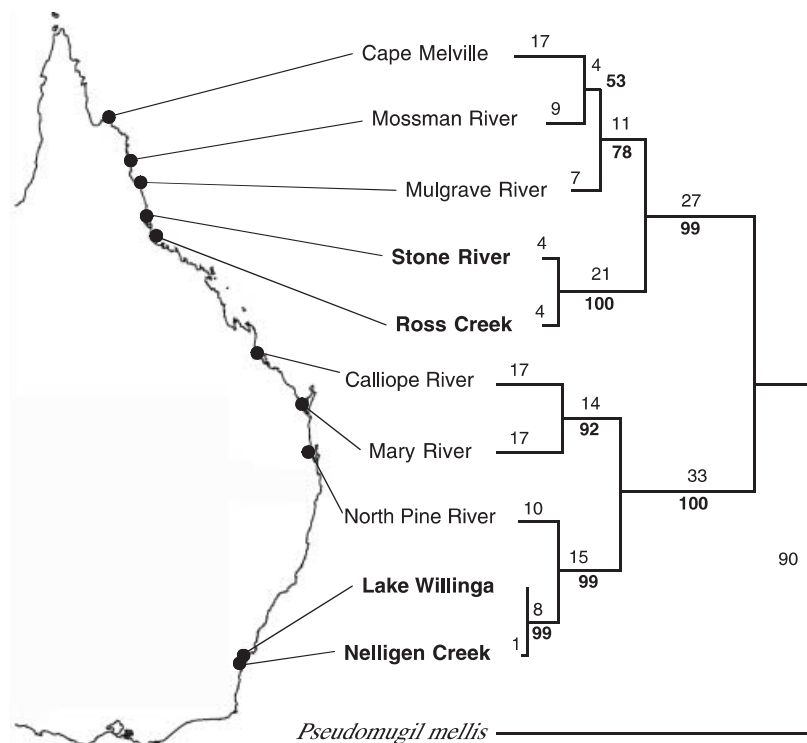

Fig. 1 Phylogram showing relationship among populations of *Pseudomugil signifer*. The branch lengths are proportional to genetic divergence. Numbers in bold are bootstrap values. All other numbers represent unweighted branch lengths.

analysis with the average linkage method based on the mean values of the natural log transformed data for each of the four populations to produce a dendrogram of morphological similarity. We also used the full natural log transformed data set in a Principle Components analysis to gain an overall picture of the amount of morphological overlap between populations.

Our clustering analysis show that the geographically and genetically close populations are also more morphologically similar to each other than they are to the geographically and genetically distant populations (Fig. 2a). LW and NC are morphologically very similar and in our PCA plot there is virtually complete overlap (Fig. 2b). RC and SR populations group together, but they are less morphologically similar to each other than the other pair (Fig. 2a), with less overlap in our PCA plot (Fig. 2b). Together, the first three principle components explained 94% of the variation (PC1: 83.75%, PC2: 6.70%; PC3: 3.56%).

Behavioural experiments

Female preferences for local and foreign males were determined in mate choice trials. Two males, each from a different population, were randomly assigned to one of two small compartments separated by an opaque divider in a 90-L aquarium (Fig. 3). Each male was supplied with his own spawning mop. A female was placed in a compartment separated from the males with a clear plexiglass divider. A spawning mop was also placed in the female's compartment to provide refuge. The aquarium set up prevented males from influencing one another but allowed the female visual access to both males. A gap surrounding the edge of the Perspex divider also permitted the possible exchange of olfactory cues between the sexes. A total of 120 spot samples were conducted over four 30-min sessions on a single day, with a minimum of 30 min between sessions. The first session took place in the morning 24 h after the introduction of fish into the aquarium. During a spot sample, a female was recorded as associating with a male if she had her body oriented unambiguously towards him whereas <5 cm in front of his compartment (Wong, in press). Females were tested once, each with a different pair of males.

The preferred male was determined as the one with whom the female had spent the most time in association. In an earlier study, this translated into actual mating preferences as females spawned significantly sooner when paired with a 'preferred' male (Wong, in press). We were also interested in the *strength* of preference. This was calculated as the percentage of time a female associated with a particular male out of the total time she spent associating with males.

Choice between local and foreign male
We tested female preference for local vs. foreign males using females from two populations, LW and RC.
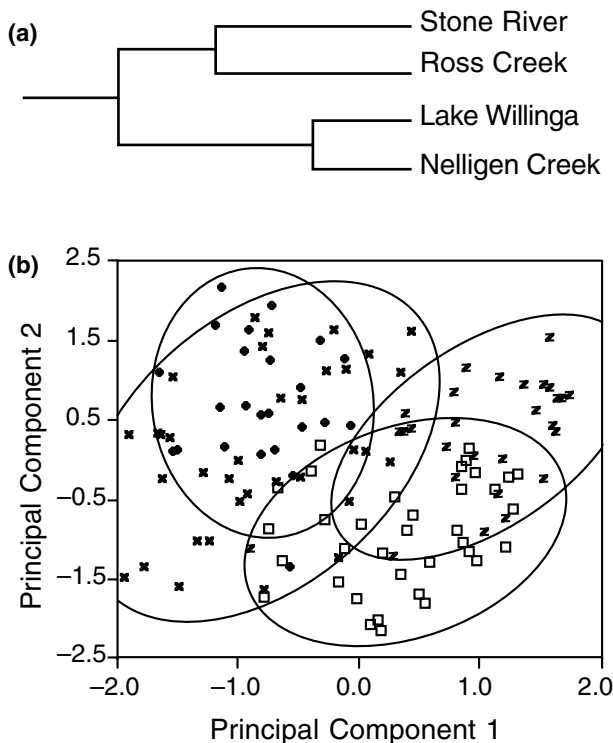

Fig. 2 Morphological similarities among the focal populations based on six morphological characters. (a) Dendrogram based on a cluster analysis. (b) Plot of PC1 by PC2 based on a principal components analysis. ● Nelligen Creek (*n* = 24), □ Stone River (*n* = 36), ✖ Lake Willinga (*n* = 36), Z Ross Creek (*n* = 30).
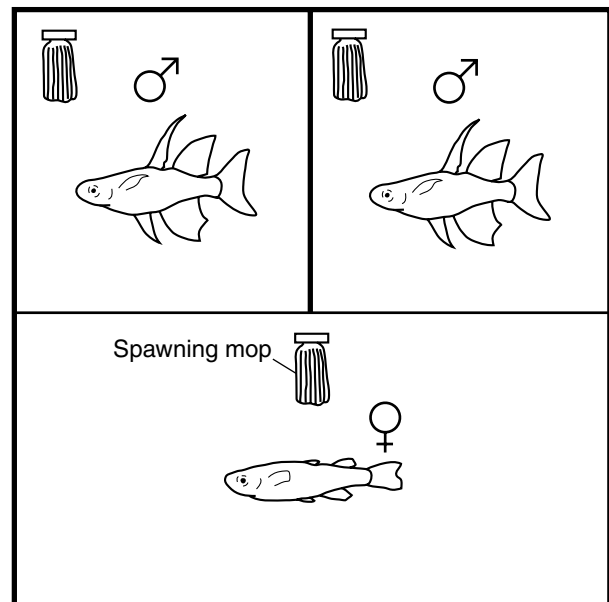

Fig. 3 Mate choice set up for behavioural experiments.

Females in each focal population were offered the choice between a male from her own population and a male from one of two allopatric populations that differed in their relative geographical and genetic distance to the focal population as follows:

LW females: LW male vs. NC male (i.e. local vs. foreign close)

LW male vs. RC male (i.e. local vs. foreign far)

RC females: RC male vs. SR male (i.e. local vs. foreign close)

RC male vs. LW male (i.e. local vs. foreign far)

Following Tilley *et al.* (1990), we predicted that more females would prefer local males. Moreover, we expected that preferences for locals should be stronger when the foreigner is from the more distant population compared with trials where he is from the geographically and genetically more proximate population. Hence, unless otherwise stated, all statistical tests are one-tailed and data is presented as mean ± SE.

In total, 69% of LW females (25 of 36) spent more time associating with local over foreign males. There was, however, a significant difference between treatments depending on the origin of the foreign male (Fisher's Exact, $P = 0.01$). Sixteen of 18 LW females preferred the local male when the alternative was a male from the distant RC population (Binomial test, $P < 0.001$). In contrast, only nine of 18 LW females preferred the local when the foreigner was a male from the more proximate NC population (Binomial test, $P = 0.5$). There also was a significant difference in the strength of female preference for local males depending on treatment (Two Sample t-test, $t = 2.46$, d.f. $= 34$, $P = 0.01$): LW females spent $70.0 \pm 6.5\%$ of their time associating with the local when the foreign male was from RC but only $45.1 \pm 7.8\%$ when the foreigner was from NC (Fig. 4). The former also differed significantly from 50% but the latter did not (One Sample t-test, $t = 3.08$, d.f. $= 17$, $P < 0.01$; $t = -0.65$, d.f. $= 17$, $P = 0.27$).

The results obtained for RC females were very similar to those obtained for LW females. In total, 67% of RC females (24 of 36) spent more time associating with local over foreign males. Again, there was also a significant difference between treatments (Fisher's Exact, $P < 0.001$). Seventeen of 18 RC females preferred the local when the alternative was a male from the more distant LW population (Binomial test, $P < 0.001$) whereas only seven of 18 RC females spent more time with the local when the alternative was a male from the more proximate SR population (Binomial test, $P = 0.24$). The strength of female preferences also differed between treatments (Two Sample t-test, $t = 1.89$, d.f. $= 34$, $P < 0.05$): RC females spent 73.5 ± 7.2 and $54.1 \pm 7.3\%$ associating with the local male when the foreign male was from LW and SR respectively (Fig. 4). The former also differed significantly from 50% but the
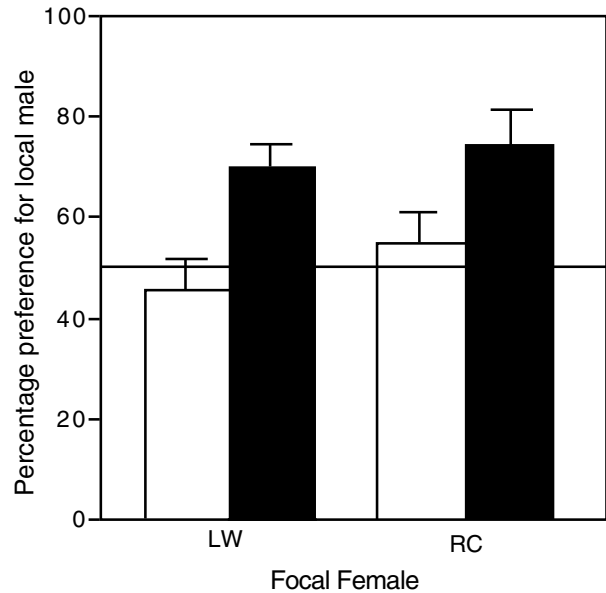

Fig. 4 Mean percentage preference ± SE for local male by Lake Willinga (LW) and Ross Creek (RC) females when the foreign male is from a geographically and genetically proximate population (open bar) and when the foreigner is from a geographically and genetically more distant population (black bar). All $n = 18$.

latter did not (One Sample t-test, $t = 3.28$, d.f. $= 17$, $P < 0.01$; $t = 0.58$, d.f. $= 17$, $P = 0.29$).

Choice between two foreign males

In this experiment we tested female preference for males from two allopatric populations that differ in their relative geographical and genetic distance to the focal female's population. Specifically, females from the two focal populations were presented with a choice between males as follows:

LW females: NC male vs. RC male (i.e. foreign close vs. foreign far)

RC females: SR male vs. LW male (i.e. foreign close vs. foreign far)

Based on the results of our previous mate choice experiments, we expected females to favour males from the population that was geographically and genetically more similar to their own. Our results were consistent with this prediction. Fourteen of 18 LW females preferred NC over RR males (Binomial test, $P < 0.05$) while 13 of 18 RC females preferred SR males over those from LW (Binomial test, $P < 0.05$). Also, in terms of strength of preference, the percentage of time spent by both LW and RC females with a male from the more proximate foreign population was significantly >50% (LW: one sample t-test, $t = 3.57$, d.f. $= 17$, $P = 0.001$, mean ± SE $= 70.9 \pm 5.8\%$; RC: one sample t-test, $t = 2.19$, d.f. $= 17$, $P < 0.05$, mean ± SE $= 68.9 \pm 8.6\%$).

Male courtship

Male blue-eyes actively court females (Wong & Jennions, 2003) and a previous study showed that females use courtship as a cue in mate choice (Wong, in press). Thus, during the choice trials described above, we scored male behaviour (courting, swimming, stationary) whenever the female was associating with a particular male. From this, we obtained the proportion of time spent courting by each male when the female was associating with him.

Overall, preferred males courted more per unit time when females were associating with them than did nonpreferred males (preferred = 32.68 ± 2.44%, nonpreferred = 24.14 ± 2.79%; Paired *t*-test, $t = 2.561$, d.f. = 107, $P = 0.012$). The relative strength of female preferences was greater for males that spent more time in courtship ($r = 0.78$, $P < 0.001$, $n = 216$).

Discussion

Studies in a range of taxa have demonstrated that females often recognize and prefer to mate with local over foreign males (e.g. snails: Rupp & Woolhouse, 1999; fish: Endler & Houde, 1995; salamanders: Tilley *et al.*, 1990; Herring & Verrell, 1996; rodents: Pillay *et al.*, 1995; Pillay, 2000). Females, however, do not always prefer homotypes. Endler & Houde (1995), for example, discovered that female guppies *P. reticulata* were significantly more attracted to local males in only five of 11 populations. Females in the remaining six populations lacked a strong preference for their own males (see also Magurran *et al.*, 1996; Houde & Hankes, 1997; Ptacek & Travis, 1997). Similarly, female blue-eyes are not always attracted to males from their own populations. Our results indicate that female preference for locals may, instead, depend on how divergent the allopatric populations are in relation to their own. Specifically, we found that female blue-eyes chose local over foreign males only when the latter was from a geographically more distant population. In contrast, locals, on average, were no more attractive than foreigners when the latter was from a proximate (50–200 km) population.

Our molecular phylogeny demonstrates strong concordance between the extent of geographical isolation among focal populations and the degree of genetic differentiation. More distantly allopatric populations appear to have undergone greater divergence in mate recognition systems associated with longer periods of genetic isolation (Tilley *et al.*, 1990; Endler & Houde, 1995). This is evidenced by the ability of females to discriminate against foreign males from a geographically and genetically more distant population, but not males from a population more proximate to their own. Signal similarities because of phylogenetic relatedness can have important implications for recognition of mates (Strecker & Kodric-Brown, 1999; de Kort & ten Cate, 2001). It is possible that females did not discriminate because of

morphological similarities between closely related populations. Our morphometric analysis shows that males from geographically and genetically more proximate populations are similar in appearance. These results are consistent with the comprehensive survey of morphological characters conducted by Hadfield *et al.* (1979) which showed clinal variation among 14 blue-eye populations across the species' range. When we offered females a choice between two foreign males, they preferred males from the nearby population. This result is consonant with the morphological and genetic similarities between neighbouring populations, and support the results of behavioural experiments when females were allowed to choose between local and foreign males.

In our study we inferred mating preferences based on female association preferences. One potential criticism, therefore, is that association preferences may not reflect actual mating intentions. This, however, is unlikely. First, in a previous breeding experiment, Wong (in press) showed that association preferences in blue-eyes translated into actual mating preferences with females spawning significantly sooner with the male that she had spent more time associating with. Secondly, the results of our experiments are also consistent with an earlier breeding experiment involving populations of Pacific blue-eyes from across the species' range (Semple, 1986). In that study, fish from two closely adjacent northern Australian populations interbred freely but fish taken from opposite ends of the species range did not, suggesting that interbreeding could occur between closely adjacent populations but not between those that are distantly allopatric as in *Desmognathus* salamanders (Tilley *et al.*, 1990).

Divergence of mate recognition systems is likely to be a product of interactions between the sexes (Rolan-Alvarez *et al.*, 1999; Almeda & Vistulo de Abreu, 2003) and male behaviours could play an important role in female preference for local over foreign males (e.g. Knight & Turner, 1999; Meffert & Regan, 2002). We found that the strength of female preferences in blue-eyes was correlated positively with the proportion of time a male spent in courtship when the female was associating with him. Male blue-eyes actively court females (Wong & Jennions, 2003) and courtship is an important cue used by females to select males that deliver fitness gains through higher egg hatching success (Wong, in press). Consequently, because females may also be engaged in mate quality assessment (Pfennig, 1998, 2000), females could end up directing their preferences towards foreign males that court them more vigorously. This is especially likely to occur if females do not distinguish between locals and foreigners. Our results show that male blue-eyes will court foreign females. However, males may not be indifferent with respect to female origin. This is because male blue-eyes also are choosy (Wong & Jennions, 2003). In this regard, males from geographically and

genetically distant population may discriminate against focal females that are foreign and therefore invest less time into courtship compared to either local males or those from geographically and genetically proximate populations. Future studies may wish to test this prediction directly by investigating male preferences when offered a choice between local and foreign females (e.g. Knight & Turner, 1999).

It is widely assumed that more distant allopatry should reflect reduced rates of contemporary gene flow and/or greater divergence in mate recognition systems (Tilley *et al.*, 1990; Endler & Houde, 1995; Simmons *et al.*, 2001). Our work on blue-eye fish supports this assumption. However, in general, close associations among these variables are often poorly tested and may not always be correct. For instance, work on grasshoppers *C. paralleus* suggest that long periods of allopatry are not essential for the evolution of substantial reproductive isolation (Tregenza *et al.*, 2000). In that study, premating isolation was found to exist between some grasshopper populations that were genetically similar while others, which were genetically and phenotypically distinct, showed no evidence of assortative mating. In another example, genetic differentiation yielded a poor fit with geographical distance in a study on stickleback *Gasterosteus aculeatus* populations in Germany (Reusch *et al.*, 2001). Rapid genetic divergence in these post-glacial populations was, instead, found to be correlated mainly with habitat type. These examples, and the results we report for blue-eye fish, suggest that future studies should more judiciously test the generality of traditional assumptions. In this regard, molecular phylogenetics, as employed in the current study, may prove to be an especially useful tool in illuminating our understanding of the forces that lead to premating reproductive isolation and speciation (Wiens *et al.*, 1999; Avise, 2000; Barraclough & Nee, 2001).

Acknowledgments

References

Almeda, C. & Vistulo de Abreu, F. 2003. Dynamic instabilities lead to sympatric speciation. *Evol. Ecol. Res.* **5**: 739–757.

Avise, J.C. 2000. *Phylogeography: The History and Formation of Species*. Havard University Press, Cambridge, MA, USA.

Barraclough, T.G. & Nee, S. 2001. Phylogenetics and speciation. *Trends in Ecology and Evolution* **16**: 391–399.

Basolo, A.L. 1995. Phylogenetic evidence for the role of a pre-existing bias in sexual selection. *Proc. R. Soc. Lond. B.* **259**: 307–311.

Blomberg, S.P. & Garland, T. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *J. Evol. Biol.* **15**: 899–910.

Boake, C.R.B. 2002. Sexual signaling and speciation, a micro-evolutionary perspective. *Genetica.* **116**: 205–214.

Castellano, S., Giacoma, C. & Dujsebayeva, T. 2000. Morphometric and advertisement call geographic variation in polyploid green toads. *Biol. J. Linn. Soc.* **70**: 341–360.

Dagley, J.R., Butlin, R. & Hewitt, G.M. 1994. Divergence in morphology and mating signals, and assortative mating among populations of *Chorthippus paralleus* (Orthoptera: Acrididae). *Evolution* **48**: 1202–1210.

Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.

Hadfield, A.J., Ivantsoff, V. & Johnston, P.G. 1979. Clinal variation in electrophoretic and morphological characters between two nominal species of the genus *Pseudomugil* (Pisces: Atheriniformes: Pseudomugilidae). *Aust. J. Mar. Freshwater Res.* **30**: 375–386.

Herring, K. & Verrell, P.A. 1996. Sexual incompatabilities and geographical variation in mate recognition systems: tests in the salamander *Desmognathus ochrophaeus*. *Anim. Behav.* **52**: 279–287.

Hill, G.E. 1994. Geographic variation in male ornamentation and female preference in the house finch: a comparative test of models of sexual selection. *Behav. Ecol.* **5**: 64–73.

Houde, A.E. & Hankes, M.A. 1997. Evolutionary mismatch of mating preferences and male colour patterns in guppies. *Anim. Behav.* **53**: 343–351.

Jukes, T.H. & Cantor, C.R. 1969. Evolution of protein molecules. In: *Mammalian Protein Metabolism* (H. N. Munro, ed.), pp. 21–132. Academic Press, New York.

Keogh, J.S., Scott, I.A.W. & Scanlon, J. 2000. Molecular phylogeny of viviparous Australian elapid snakes: affinities of 'Echiopsis' atriceps (Storr, 1980) and 'Drysdalia' coronata (Schlegel, 1837), with description of a new genus. *J. Zool., Lond.* **252**, 317–326.

Knight, M.E. & Turner, G.F. 1999. Reproductive isolation among closely related Lake Malawi cichlids: can males recognize conspecific females by visual cues? *Anim. Behav.* **58**: 761–768.

de Kort, S.R. & ten Cate, C. 2001. Response to interspecific vocalisations is affected by degree of phylogenetic relatedness in *Streptopelia* doves. *Anim. Behav.* **61**: 239–247.

Magurran, A.E., Paxton, C.G.M., Seghers, B.H., Shaw, P.H. & Carvalho, G.R. 1996. Genetic divergence, female choice and male mating success in Trinidadian guppies. *Behaviour* **133**: 503–517.

Masta, S.E. & Maddison, W.P. 2002. Sexual selection driving diversification in jumping spiders. *Proc. Natl. Acad. Sci. USA* **99**: 4442–4447.

McGlashan, D.J. & Hughes, J.M. 2002. Extensive genetic divergence among populations of the Australian freshwater fish, *Pseudomugil signifer* (Pseudomugilidae), at different hierarchical scales. *Mar. Freshwater Res.* **53**: 1–11.

Meffert, L.M. & Regan, J.L. 2002. A test of speciation via sexual selection on female preferences. *Anim. Behav.* **64**: 955–965.

Mendelson, T.C. & Shaw, K.L. 2002. Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (Orthoptera: Gryllidae). *Genetica.* **116**: 301–310.

Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.

Pfennig, K.S. 1998. The evolution of mate choice and the potential conflict between species and mate-quality recognition. *Proc. R. Soc. Lond. B.* **265**: 1743–1748.

Pfennig, K.S. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behav. Ecol.* **11**: 220–227.

Pillay, N. 2000. Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio. Behaviour* **137**: 1431–1441.

Pillay, N., Willan, K. & Meester, J. 1995. Evidence of pre-mating reproductive isolation in two allopatric populations of the vlei rat (*Otomys irroratus*). *Ethology* **100**: 61–71.

Ptacek, M. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. *Behav. Proc.* **51**: 111–134.

Ptacek, M.B. & Travis, J. 1997. Mate choice in the Sailfin Molly, *Poecilia latipinna. Evolution* **51**: 1217–1231.

Reusch, T.B.H., Wegner, K.M. & Kalbe, M. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. *Mol. Ecol.* **10**: 2435–2445.

Rolan-Alvarez, E., Erlandsson, J., Johannesson, K. & Cruz, R. 1999. Mechanisms of incomplete prezygotic reproductive isolation in an intertidal snail: testing behavioural models in wild populations. *J. Evol. Biol.* **12**: 879–890.

Rupp, J.C. & Woolhouse, M.E.J. 1999. Impact of geographical origin on mating behaviour in two species of *Biomphalaria* (Planorbidae: Gastropoda). *Anim. Behav.* **58**: 1247–1251.

Ryan, M.J. & Rand, A.S. 1990. The sensory basis of sexual selection for complex calls in the tungara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). *Evolution* **44**: 305–314.

Semple, G. 1986. *Pseudomugil signifer* – maintenance, reproduction and early development of the Pacific blue-eye. *Fishes of Sahul* **3**: 121–125.

Shaw, K.L. 2000. Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. *Evolution* **54**: 1303–1312.

Simmons, L.W., Zuk, M. & Rotenberry, J.T. 2001. Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus. Evolution* **55**: 1386–1394.

Strecker, U. & Kodric-Brown, A. 1999. Mate recognition systems in a species flock of Mexican pupfish. *J. Evol. Biol.* **12**: 927–935.

Swofford, D.L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, Version 4. Sinauer Associates, Sunderland, MA, USA.

Tilley, S.G., Verrell, P.A. & Arnold, S.J. 1990. Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochrophaeus. Proc. Natl. Acad. Sci. USA* **87**: 2715–2719.

Tregenza, T., Pritchard, V.L. & Butlin, R. 2000. The origins of premating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus. Evolution* **54**: 1687–1698.

Wiens, J.J., Reeder, T.W. & De Oca, A.N.M. 1999. Molecular phylogenetics and evolution of sexual dichromatism among populations of the Yarrow's spiny lizard (*Sceloporus jarrovii*). *Evolution* **53**: 1884–1897.

Wong, B.B.M. in press. Superior fighters make mediocre fathers in the Pacific blue eye fish. *Anim. Behav.*

Wong, B. & Jennions, M.D. 2003. Costs influence male mate choice in a freshwater fish. *Proc. R. Soc. Lond. B (Suppl.), Biol. Lett.* **270**: 36–39.

Wong, B.B.M., Keogh, J.S. & McGlashan, D.J. 2004. Current and historical patterns of drainage connectivity in eastern Australia inferred from genetic structuring in a widespread freshwater fish. *Pseudomugil signifer* (Pseudomugilidae) *Mol. Ecol.* **13**: 391–401.