

## Evaluation of Female Mate Choice Cues in a Group of Lake Malawi *Mbuna* (Cichlidae)

REBECCA JORDAN, KAREN KELLOGG, FRANCIS JUANES, AND JAY STAUFFER JR.

The mechanisms that underlie the diversity of cichlids in the East African Great Lakes are poorly understood. Sexual selection through female choice based on male body coloration has often been suggested as a driving force behind the speciation of these fishes. The objectives of this study were to investigate, through mate choice trials, the cues that guide species-isolating female choice. In a group of sympatric Lake Malawi *mbuna* (rock-dwelling fish), we investigated both visual and chemical cues that might guide female choice by giving gravid females a choice between a heterospecific and a conspecific male. Visual cues, in contrast to olfactory cues, were sufficient to stimulate courtship and thus guide female choice of males. Furthermore, in contrast to other studies on related species, we found that females courted only with conspecifics even if color was not a cue. Species-isolating female choice is likely based primarily on visual information.

THE cichlid fishes of the African Great Lakes are the most extreme case of adaptive radiation among vertebrates. An example of this astounding diversification is the monophyletic group of at least 700 cichlid species that has evolved in Lake Malawi over the past 2 million years (Turner, 2001). When explaining this rapid rate of speciation in Lake Malawi, most researchers agree that ecological mechanisms abetted by several cichlid characteristics including habitat fidelity, low rates of dispersion, and plasticity of the feeding apparatus (Fryer and Iles, 1972; Liem, 1974; Danley and Kocher, 2001) are important. In addition, large lake level fluctuations have created ephemerally isolated habitats (Scholz and Rosendahl, 1988), which can increase the opportunities for speciation given that cichlid fish are often philopatric.

Certain speciation events in Lake Malawi, such as those among closely related groups, are not associated with detectable changes in ecology or morphology (excluding color: Albertson et al., 1999; Genner et al., 1999). In these groups, population isolation and ultimately speciation may best be linked to sexual selection (Dominey, 1984; Knight et al., 1998; Couldridge and Alexander, 2002). The majority of Lake Malawi cichlids have life history characteristics that predispose them to sexual selection through female choice, including polygynous mating systems, lekking behavior in males, and exclusive female parental care (Fryer and Iles, 1972; Andersson, 1994). We know very little, however, about the cues that Lake Malawi females use to select among males of closely related species (i.e., species-isolating mate choice).

To study species-isolating female choice, we

focused on the *mbuna* (rock-dwelling cichlids), a group of over 200 species (Danley and Kocher, 2001). Overall, the *mbuna* are behaviorally and morphologically similar, although color varies dramatically among species and is especially brilliant in courting males (Fryer and Iles, 1972). Furthermore, the *mbuna* species are reproductively isolated. Even though many species are sympatric, closely related, and in many cases use the same breeding arenas, they interbreed very little in the field (van Oppen et al., 1998) or in the laboratory (Knight et al., 1998).

There is limited, indirect evidence to support the importance of vision in *mbuna* interspecies choice. Male body coloration differs markedly among closely related species, whereas females tend to look very similar to each other (Deutsch, 1997; McElroy et al., 1991). Furthermore, when given a choice of potential mates presented only through visual cues, some female *mbuna* interacted preferentially with conspecific males or, when a conspecific male was not present, the same females interacted with those males that were most similar in color to their conspecifics (Couldridge and Alexander, 2002). In that study, however, the role of male body coloration was never directly tested, making it difficult to assess its importance as a mate choice cue.

In addition, nonvisual cues remain untested. There is evidence for species-specific sound production in Lake Malawi cichlids (Lobel, 1998) and for the use of pheromones in mate selection in other African cichlids (Crapon de Caprona, 1974). We, therefore, investigated both visual (including color) and nonvisual cues that might be used by females during species-isolat-

ing mate choice in a group of sympatric Lake Malawi *mbuna*.

#### MATERIALS AND METHODS

*Housing and fish.*—We used three sympatric species for our experiments: *Metriaclima zebra* (males are blue with black bars), *Metriaclima benetos* (males are primarily blue); and *Metriaclima barlowi* (males are yellow with blue anal fin). Although males of the three species vary substantially in color and pattern, the females are all solid brown with the exception of additional faint vertical bars in female *M. zebra* (for pictures, see [www.princeton.edu/~jordan/pictures.htm](http://www.princeton.edu/~jordan/pictures.htm)). These fish were collected from depths of approximately 3–8 m at Mazinzi reef, an approximately 200 m<sup>2</sup> rocky area located along the west coast of the southeastern arm of Lake Malawi (14S–35E).

All fish were housed in 110-liter aquaria (with occasional, short-term separation of brooding females or injured fish). Each aquarium was maintained at 24–28 C, pH 7–8, and 12-h light/dark cycles. The fish were given a combination of commercially available flake and stick food three times daily. Except for feeding and tank maintenance, the fish were not disturbed.

*Mate choice experiments.*—Gravid females (those having swollen ventral and urogenital regions) were presented a choice of two similarly sized (within 4 mm total length, TL) males, one conspecific and one heterospecific. We used only fish that were comparable in size to field specimens (i.e., males were 12–15 cm TL, and females ranged between 9.5 and 11 cm TL; for field descriptions, see Konings, 1990). We used females that were slightly smaller than the males to avoid any aggressive interactions.

The tank system (188 liters) for all mate choice trials included a central compartment for the female (94 liters) and two lateral compartments, one for each male (47 liters each). Depending on the treatment, the compartments were separated by either a glass partition or opaque perforated partitions. Two additional opaque partitions, each spanning two-thirds of the width of the tank, were offset within the center compartment to eliminate male-male visual contact while allowing a female to move to and from areas adjacent to males. In all experiments, a heater was placed in the center of the female compartment, and air stones at maximum power were placed directly center and back in all compartments. Lighting (except for the monochromatic treatment) consisted of four broad-spectrum bulbs (2 daylight simulat-

ing incandescent radiation containing at least 4% UV-A radiation and 2 fluorescent UV-enhanced bulbs containing 7% UV-A, and 3% UV-B radiation) mounted directly over the test tank.

For each experiment, two heterospecific males were selected, and one male was placed in each lateral compartment. After allowing the males to acclimate for six hours, a female conspecific of one of the males was introduced to the center of the tank and allowed to acclimate for 15 min. We then videotaped the female's behavior for 45 min. Forty-five minutes is ample time to observe choice in gravid females (Couldridge and Alexander, 2001).

Female behavior was categorized as either interactive or noninteractive. A female was interacting with a particular male if she was in the portion of her compartment that was between the male/female separating partition and the opaque blocking partition and engaged in any of the following: approaching the male/female separating partition with her snout close to or touching the partition; rapid swimming in the proximity of the male/female partition; or swimming in circles either to and from the male/female separating partition or along side that partition. In most cases, males performed these interactive behaviors synchronously with the female and with the addition of quivering the anal fin.

We used Wilcoxon signed rank tests to compare proportions of interaction times of all the females tested in a particular experiment. Proportion of interaction time is the ratio of interaction time with an individual male to the total interaction time, where total interaction time is the sum of interaction time spent with both males. The use of female behavior prior to contact with the males to predict female choice has been verified in previous studies (Kellogg, 1997; Couldridge and Alexander, 2001).

In all nonvisual experiments, females failed to engage in interactive behavior. Although not overt, we did not want to rule out the possibility that females were still associating with the conspecific male. In these cases, we compared, using the Wilcoxon signed rank test, proportion of association time. Association time is the time that a female spends, regardless of her behavior, in the region of her compartment that is separated by the male/female separating partition and the opaque partition. Similar to the analysis described above, proportion of association time is the ratio of association time with a given male to the total association time, and total association time is the sum of association time spent with both males.

Because mortality reduced the number of available males over time, approximately 15% were used more than once in an experiment. Each pair, however, was unique because no male was paired with the same male twice. Females were used successively in the four different experiments (i.e., in order, visual-broad spectrum, olfactory-same tank, olfactory-different tank, and visual-monochromatic) but were not used repeatedly within an experiment.

*Visual-broad spectrum experiments.*—To determine whether females interacted assortatively with conspecific males in the presence of visual but no chemical or auditory cues, we gave females a choice between a conspecific and a heterospecific male behind transparent solid glass partitions. The crown glass used had a spectral transmittance of at least 80% at 350 nm (into the ultraviolet spectral region). To simulate natural daylight conditions, we used the broad-spectrum lighting described above.

*Visual-monochromatic experiments.*—To determine whether coloration of the visual signal facilitates choice, females were given a choice between a conspecific and a heterospecific male under monochromatic lighting. For this experiment, we retained the crown glass partitions from the previous experiment. We placed both “plasma” red and “medium” red (Lee Filters, Inc.) filters with peak transmissions above 600 nm over the broad-spectrum lighting. When used simultaneously, these filters effectively block all color wavelengths except red. Because these fish have no red coloration and only minimal sensitivity to red (R. C. Jordan, K. A. Kellogg, F. Juanes, J. R. Stauffer Jr., and E. R. Loew, unpubl.), color differences between the males would be masked.

*Olfactory-same tank experiments.*—To determine whether chemical cues (with sound) in the absence of visual information would result in assortative mating, females were first given a choice between a conspecific and a heterospecific male behind opaque partitions. These partitions blocked vision but passed potential chemical cues through a 5 mm grid of 1 mm perforations. To further ensure visual separation of fish, each partition was constructed of two slightly offset perforated layers. Aeration via air stones was provided to facilitate exchange of chemical cues through the partitions. Water exchange was tested using red dye.

Absence of female interactive behavior in eight initial trials led us to hypothesize that chemical cues may only be released during

courtship. To test this, we conducted conspecific-only, olfactory-same tank experiments to compare potential differences in female reaction to courting and noncourting males. Females were given a choice between a solitary conspecific male and a conspecific male that had visual access to a conspecific female in a separate tank

*Olfactory-different tank experiments.*—Because the heterospecific olfactory-same tank treatment did not control for sound and also unrealistically eliminated all visual access of males to females, we conducted trials in which males were kept in separate tanks from both each other and the test female but were given visual access to peripheral conspecific females in separate tanks. The presence of these females stimulated male courting behavior. Water potentially containing chemical cues was collected from these separate male tanks and released via medical intravenous (IV) tubing (4 mm diameter) at a rate of a single drop per second into the water on either side of the perforated partitions.

## RESULTS

*Visual-broad spectrum experiments.*—Under broad-spectrum lighting, females spent significantly more time interacting with their own species than with conspecifics (Wilcoxon test:  $T_{0.05} = 144$ ,  $n = 17$ ,  $P = 0.001$ ; Fig. 1). Visual information appeared sufficient for assortative mating with conspecifics. In these trials both males and females engaged in interactive behavior.

*Visual-monochromatic experiments.*—Under monochromatic red lighting, again females spent significantly more time interacting with conspecifics (Wilcoxon test:  $T_{0.05} = 21$ ,  $n = 8$ ,  $P = 0.036$ ). Although color was obscured under the monochromatic light, pattern and shape were not. Simply obscuring color information was not sufficient to break down reproductive isolation. In these trials, females and males engaged in interactive behavior.

*Olfactory-same tank experiments.*—When females were given a choice between heterospecific and conspecific males behind the perforated partitions, females did not exhibit interactive behavior. Because of this, we used association time and not interaction time for our statistical analysis. Females did not spend significantly more time in the region of either male (Wilcoxon test:  $T_{0.05} = 13$ ,  $n = 8$ ,  $P = 0.441$ ; Fig. 2). When given a choice of unseen conspecific males in which one male was actively courting another female, females again did not spend significant

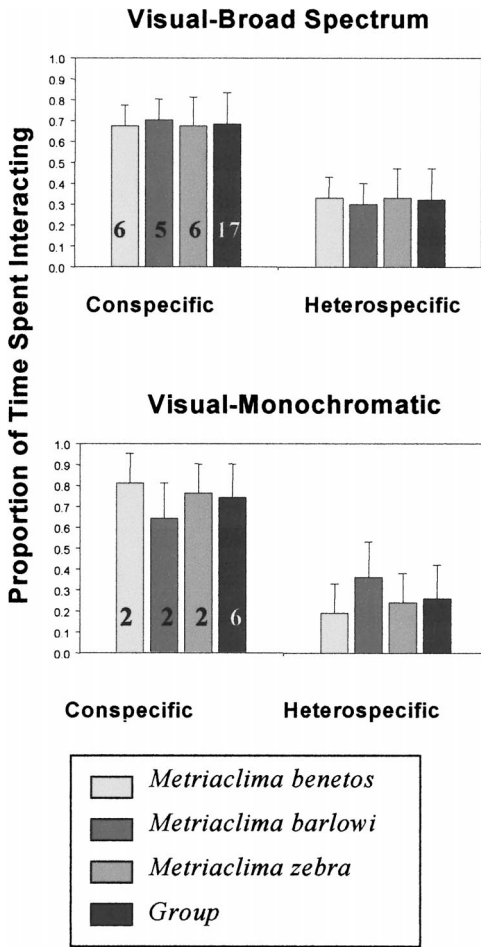


Fig. 1. Average proportion of interaction time spent with either a con- or heterospecific male in visual experiments. Within an experiment, the data are presented as a group and by species. The sample sizes are marked on the bars. Only the visual experiments yielded significant differences between the heterospecific and conspecific males according to a Wilcoxon signed rank test,  $\alpha = 0.05$ .

ly more time with one male versus the other (Wilcoxon test:  $T_{0.05} = 11$ ,  $n = 8$ ,  $P = 0.529$ ). Thus, chemical cues in the presence of sound were not sufficient to stimulate courtship interaction or to cue females to their own species. Again, neither the female nor the males engaged in interactive behavior.

*Olfactory-different tank experiments.*—Finally, when females were given a choice between water containing potential chemical cues from courting conspecific and heterospecific males, females again exhibited no interactive behavior and did not spend any more time associating with one male versus the other (Wilcoxon test:  $T_{0.05} = 9$ ,

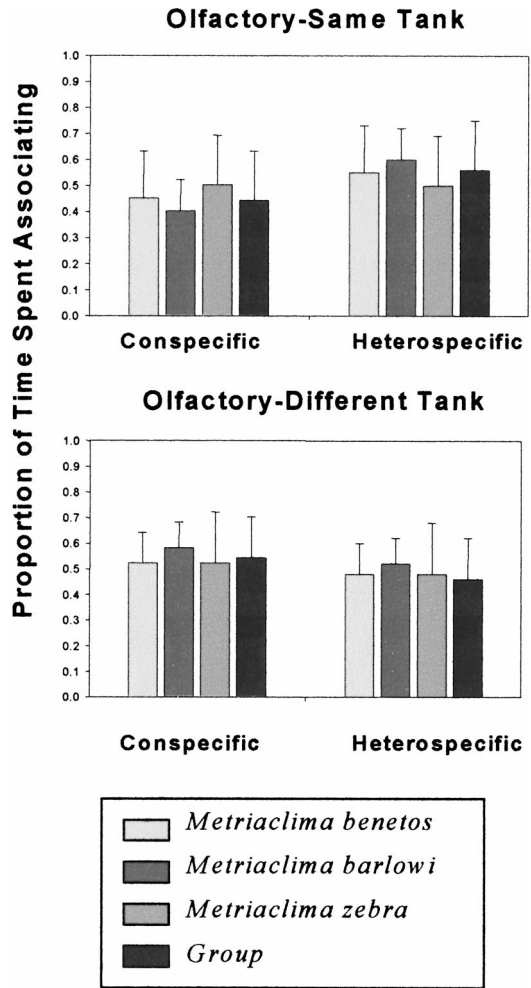


Fig. 2. Average proportion of association time spent with either a con- or heterospecific male in olfaction experiments. Within an experiment, the data are presented as a group and by species. The sample sizes are marked on the bars. None of the experiments yielded significant differences between the heterospecific and conspecific males according to a Wilcoxon signed rank test,  $\alpha = 0.05$ .

$n = 8$ ,  $P = 0.834$ ). Again, chemical cues alone were not sufficient to stimulate interaction.

We pooled species for analysis after examining similarity in responses among species. The three species were similar in average proportion of interaction and association time spent with conspecifics versus heterospecifics (Figs. 1–2). Within the visual experiments, we found no significant differences among the three species in their average total interaction times (one-way ANOVA  $P = 0.842$ ,  $F = 0.2$ ,  $n = 17$ ; six *M. benetos*, six *M. zebra*, and five *M. barlowi*). In the nonvisual trials, we found no difference in the

average total association times (one-way ANOVA,  $P = 0.364$ ,  $F = 1.1$ ,  $n = 8$ ; three *M. benetos*, two *M. zebra*, and three *M. barlowi*). Therefore, the three species not only responded similarly among the trials within the four experiments but also spent an equal amount of time responding. Nearly equal numbers of females from each species were used in each experiment (Figs. 1–2), it is unlikely that the response of any species would mask the response of the other two species.

#### DISCUSSION

Male body coloration may be an initially diverging characteristic used for reproductive isolation (Seehausen et al., 1999b). Color is a rapidly evolving characteristic (Deustch 1997, Seehausen et al., 1999a) that is under relatively simple genetic control compared to other morphological characters (Kornfield, 1991). Previous studies on Lake Malawi cichlids and other fishes demonstrate the importance of male body coloration in the maintenance of species isolation (Seehausen et al., 1997; Boughman, 2001; Couldrige and Alexander, 2002).

Visual cues are important for species recognition in *mbuna*, but color itself may not be. Although the monochromatic light used in the second set of our visual experiments obscured color, it did not obscure differences in behavior, shape, and pattern of males. The only comprehensive study of courtship behavior in *mbuna* (McElroy and Kornfield 1990) found no significant difference in courtship patterns among the nine species of males, including the three species tested in this study. The species that we used are similar in overall body shape, although *Metriaclima barlowi* males have a slightly differently shaped head and snout than do male *M. benetos* and *M. zebra* (JS, unpubl. data). Only *M. zebra* males have a distinct barring pattern. *Metriaclima benetos* develops a barring pattern similar to *M. zebra* males but lose the bars during courtship (RJ, unpubl. data). Given these pattern and shape differences, it is likely that females could discern among the three species in the absence of color.

The ability to distinguish among males using noncolor visual cues may also exist in the group of female *mbuna* tested by Couldrige and Alexander (2002). Their study found that each female, in the absence of conspecifics, associated with the heterospecific male most similar in color to her conspecific male. It is not clear, however, whether these females could distinguish between heterospecifics and conspecifics under monochromatic lighting as the females did in

our study. Although color may influence choice among heterospecifics, it may not be necessary to species recognition in the presence of other cues, such as pattern and shape.

In contrast to our findings, interspecific female choice based solely on body coloration has been well documented in a group of Lake Victoria cichlids (Seehausen et al., 1997, 1998) that are closely related to the cichlids of Lake Malawi. When the Lake Victoria fish were given a choice of mates under monochromatic light, females were unable to discriminate between conspecifics and heterospecifics, although they did choose conspecifics under broad-spectrum lighting. The different species of males used in that study, however, were more similarly shaped and patterned than the fish used in our study. Although both Lake Malawi and Lake Victoria fish are diverse in coloration, Lake Malawi fish are more diverse in shape (Barel, 1984), and the genetic divergence among Lake Malawi species is greater (Meyer, 1993). Lake Malawi is almost 2 million years old, whereas Lake Victoria is much younger at 14 thousand to 700 thousand years old (Johnson et al., 1996). Lake-specific differences may relate to differences in the time required to evolve color versus behavioral or morphological isolating mechanisms

#### ACKNOWLEDGMENTS

This work was supported by the Graduate Assistance in Areas of National Need (GAANN) Fellowship Program through the Graduate Program in Organismic and Evolutionary Biology from the Federal Department of Education, the American Cichlid Association, and a University of Massachusetts Healy Grant. The University of Massachusetts Institutional Animal Care and Use Committee have approved the methods used in this work under protocol number 19–02–03. We would like to thank the Malawi government for making this work possible. We also wish to thank J. Tobey, C. Vasconcelos, and D. Howe for their help on this project, B. Byers, S. Pratt, A. McKnight, our section editor, and two anonymous reviewers for their thoughtful reviews, and E. Loew for his advice. Finally, we acknowledge the University of Massachusetts where this work was conducted.

#### LITERATURE CITED

- ANDERSSON, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.
- BAREL, C. D. N. 1984. Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). Neth. J. Zool. 33:357–424.

- BOUGHMAN, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948.
- COULDRIDGE, V. C. K., AND G. J. ALEXANDER. 2001. Does the time spend near a male predict female mate choice in a Malawian cichlid? *J. Fish Biol.* 59: 667–672.
- , AND ———. 2002. Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behav. Ecol.* 13:59–64.
- CRAPON DE CAPRONA, M. D. 1974. The effect of chemical stimuli on the behavior of *Haplochromis burtoni* (Cichlidae, Pisces). *Experientia* 30:1394–1395.
- DANLEY, P., AND T. KOCHER. 2001. Speciation in rapidly diverging systems: lessons from Lake Malawi. *Mol. Ecol.* 10:1075–1086.
- DEUTSCH, J. C. 1997. Color diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? *Biol. J. Linn. Soc.* 62:1–14.
- DOMINEY, W. J. 1984. Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. p. 231–249. *In: Evolution of fish species flocks*. A. A. Echelle and I. Kornfield (eds.). Univ. of Maine Press, Orono.
- FRYER, G., AND T. D. ILES. 1972. The Cichlid fishes of the Great Lakes of Africa: their biology and evolution. Oliver and Boyd, Edinburgh, Scotland.
- GENNER, M. J., G. F. TURNER, AND S. J. HAWKINS. 1999. Foraging of rocky habitat cichlid fishes in Lake Malawi: coexistence through niche partitioning. *Oecologia* 121:283–292.
- JOHNSON, T. C., M. R. SCHOLZ, K. TALBOT, R. D. KELTS, G. RICKETTS, K. NGOBI, I. BEUNIG, I. SSEMMANDA, AND J. W. MCGILL. 1996. Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science* 273:1091–1093.
- KELLOGG, K. A. 1997. Lake Malawi cichlid mating systems: factors that influence mate selection. Pennsylvania State Univ., University Park.
- KNIGHT, M. E., AND G. F. TURNER, C. RICO, M. J. H. VAN OPPEN, AND G. M. HEWITT. 1998. Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Mol. Ecol.* 7:1605–1610.
- KONINGS, A. 1990. *Ad Konings's book of Cichlids and all the other fishes of Lake Malawi*. TFH Publishing, Neptune, NJ.
- KORNFIELD, I. R. 1991. Genetics, p. 103–128. *In: Cichlid fishes: behavior, ecology, and evolution*. M. H. A. Keenleyside (ed.). Chapman and Hall, New York.
- LIEM, K. F. 1974. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst. Zool.* 22:425–441.
- LOBEL, P. S. 1998. Possible species specific courtship sounds by two sympatric cichlid fishes in Lake Malawi, Africa. *Environ. Biol. Fish.* 52:443–452.
- MCELROY, D. M., AND I. KORNFIELD. 1990. Sexual selection, reproductive behavior, and speciation in the *mbuna* species flock of Lake Malawi (Pisces: Cichlidae). *Ibid.* 28:273–284.
- MEYER, A. 1993. Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trend. Ecol. Evol.* 8:279–285.
- SCHOLZ, C. A., AND B. R. ROSENDAHL. 1988. Low lake stands in Lakes Malawi and Tanganyika, East Africa, delineated with multifold seismic data. *Science* 240: 1645–1648.
- SEEHAUSEN, O. V., J. M. VAN ALPHEN, AND F. WITTE. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Ibid.* 277:1808–1811.
- , J. M. JACQUES, AND J. M. VAN ALPHEN. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* 42:1–8.
- , P. J. MAYHEW, AND J. M. VAN ALPHEN. 1999a. Evolution of color patterns in East African cichlid fish. *J. Evol. Biol.* 12:514–534.
- , J. M. VAN ALPHEN, AND F. WITTE. 1999b. Can ancient colour polymorphisms explain why some cichlid lineages speciate rapidly under disruptive sexual selection. *Bel. J. Zool.* 129:43–60.
- TURNER, G. F., O. SEEHAUSEN, M. E. KNIGHT, C. J. ALEXANDER, AND R. L. ROBINSON. 2001. How many species of cichlid fishes are there in African Lakes. *Mol. Ecol.* 10:793–806.
- VAN OPPEN, M. J. H., G. F. TURNER, C. RICO, R. L. ROBINSON, J. D. DEUTSCH, M. J. GENNER, AND G. M. HEWITT. 1998. Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Ibid.* 7:991–1001.
- (RJ, FJ) PROGRAM IN ORGANISMIC AND EVOLUTIONARY BIOLOGY, UNIVERSITY OF MASSACHUSETTS AMHERST, AMHERST, MASSACHUSETTS 01003; (KK) ENVIRONMENTAL STUDIES PROGRAM, SKIDMORE COLLEGE, SARATOGA SPRINGS, NEW YORK 12866; AND (JS) SCHOOL OF FOREST RESOURCES, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA 16802. PRESENT ADDRESS: (RJ) DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY, GUYOT HALL, PRINCETON UNIVERSITY, PRINCETON, NEW JERSEY 08544. E-mail: (RJ) jordan@princeton.edu. Send reprint requests to RJ. Submitted: 28 Feb. 2001. Accepted: 23 Aug. 2002. Section editor: W. L. Montgomery.