subsequent induction of p34^{cdc2}, but p34^{cdc2} expression was not required for induction of c-*myb* or c-*myc*; the inhibition of p34^{cdc2} expression with antisense oligomers did not affect induction of either proto-oncogene. Thus, p34^{cdc2} expression occurs relatively late in the sequence of G$_1$-S control genes, subsequent to the induction of c-*myb* and c-*myc*. These results suggest that some of the known requirement for c-*myc* and c-*myb* for the G$_1$-S transition in lectin-stimulated T cells (*16*) could be mediated through induction of p34^{cdc2}, because reduction of p34^{cdc2} expression, either directly by antisense *cdc2* oligomers or indirectly by blocking either c-*myc* or c-*myb* induction, produces equivalent inhibition of DNA synthesis. The observed degree of inhibition of p34^{cdc2} expression after treatment of cells with antisense c-*myb* or c-*myc* could, by itself, be sufficient to block cells at G$_1$-S. However, c-*myb* and c-*myc* likely regulate the expression of many genes in G$_1$ and may control several parallel events that are also required for the G$_1$-S transition.

Our antisense oligomer experiments indicate that p34^{cdc2} is not likely to have a regulatory function at G$_0$-G$_1$ in lectin-activated T cells. The small amount of p34^{cdc2} present in resting T cells may be characteristic of G$_0$ cells, and a similar finding has also been reported for serum-starved fibroblasts (*4*) and quiescent baby rat kidney cells (*23*). It is possible, however, that p34^{cdc2} plays some role in G$_0$ cells, because expression of *cdc2* has been described in nonproliferating brain cells (*22*).

Finally, it is worth considering that aberrant expression of c-*myc* and c-*myb* in hematopoietic cells can result in leukemia and that loss of expression of Rb is associated with transformation of many cell lineages, including T cell leukemia (*24*). It is therefore possible that p34^{cdc2} itself or regulators of p34^{cdc2} function at G$_1$-S could contribute to transformation of hematopoietic cells.

REFERENCES AND NOTES

1. D. Arion *et al*, *Cell* **55**, 371 (1988); J. Gautier *et al.*, *ibid.* **53**, 433 (1988); J.-C. Labbe *et al.*, *Nature* **335**, 251 (1988).
2. G. Draetta and D. Beach, *Cell* **54**, 17 (1988).
3. G. Draetta *et al.*, *Nature* **336**, 738 (1988); V. Simanis and P. Nurse, *Cell* **45**, 261 (1986).
4. M. G. Lee *et al.*, *Nature* **333**, 676 (1988).
5. W. Dunphy and J. Newport, *Cell* **58**, 181 (1989); J. Gautier, T. Matuskawa, P. Nurse, J. Maller, *Nature* **339**, 626 (1989); K. Gould and P. Nurse, *ibid.* **342**, 39 (1989); A. O. Morla, G. Draetta, D. Beach, J. Y. J. Wang, *Cell* **58**, 193 (1989).
6. P. Nurse and Y. Bissett, *Nature* **292**, 558 (1981); S. I. Reed, J. A. Hadwiger, A. T. Lorincz, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4055 (1985).
7. A. Giordano *et al.*, *Cell* **58**, 981 (1989).
8. K. Riabowol *et al.*, *ibid.* **57**, 393 (1989).
9. S. A. Cannistra and J. D. Griffin, *Sem. Hematol.* **25**, 173 (1988); T. P. Stossel, *N. Engl. J. Med.* **290**, 717 (1974).
10. G. R. Crabtree, *Science* **243**, 355 (1989).
11. A 2.0-kb Bam HI fragment containing the human *cdc2* gene was excised from pOB231 (*26*) and inserted into the polylinker of pBD52 (B. Druker, unpublished data) to generate pSAF1. pBD52 is a derivative of pGC1 [R. M. Myers, L. S. Lerman, T. Maniatis, *Science* **229**, 242 (1985)]. pSAF1 was digested with Kpn I and Pvu II and the 0.9-kb fragment of human *cdc2* was used as a probe for Northern blotting after labeling with ^{32}P [A. P. Feinberg and B. Vogelstein, *Anal Biochem.* **132**, 6 (1983)].
12. A 23.5-kD protein fragment encompassing the COOH-terminus of p34^{cdc2} (beginning at methionine residue 85) was expressed in bacteria from the *tac* promoter. Protein was isolated by SDS–polyacrylamide gel electrophoresis and used to inoculate New Zealand White rabbits (M. Lee and H. Piwnica-Worms, unpublished data).
13. Purified p13 was isolated from an overexpressing strain of *Escherichia coli* as described [L. Brizuela, G. Draetta, D. Beach, *EMBO J.* **6**, 3507 (1987)] and coupled to cyanogen bromide–activated Sepharose 6B (5 mg per milliliter of gel) (Pharmacia).
14. A 2.0-kb Bam HI fragment containing the human *cdc2* gene was excised from pOB231 (*10*) and inserted into the Bam HI site of the baculovirus expression vector pVL941 [A. V. Luckow and M. D. Summers, *Bio/Technology* **6**, 47 (1988)]. Recombinant virus encoding p34^{cdc2} was plaque-purified and used to infect *Spodoptera frugiperda* (Sf9) cells. All procedures relating to viral propagation, isolation, and infection were performed essentially as described [M. D. Summers and G. E. Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Texas Agricultural Experiment Station Bulletin No. 1555, College Station, TX, 1987)]. Five percent of total cell protein was p34^{cdc2}, the majority of which was soluble and unphosphorylated (L. Parker and H. Piwnica-Worms, unpublished data).
15. P. Kurki *et al.*, *J. Immunol.* **138**, 4114 (1987).
16. R. Heikkila *et al.*, *Nature* **328**, 445 (1987); A. M. Gewirtz *et al.*, *Science* **245**, 180 (1989); A. M. Churilla *et al.*, *J. Exp. Med.* **170**, 105 (1989).
17. J. A. DeCaprio *et al.*, *Cell* **58**, 1085 (1989); K. Buchkovich, L. A. Duffy, E. Harlow, *ibid* , p. 1097; P.-L. Chen *et al* , *ibid.*, p. 1193; K. Mihara *et al.*, *Science* **246**, 1300 (1989); H.-J. Xu *et al.*, *Oncogene* **4**, 807 (1989).
18. Y. Furukawa *et al.*, *Proc. Natl. Acad Sci. U.S.A.* **87**, 2770 (1990).
19. Y. Taya *et al.*, *Biochem. Biophys. Res. Commun.* **164**, 580 (1989).
20. J. A. DeCaprio *et al.*, *Cell* **54**, 275 (1988); J. W. Ludlow *et al.*, *ibid.* **56**, 57 (1989); J. W. Ludlow *et al.*, *ibid.* **60**, 387 (1990).
21. K. Kelley, B. H. Cochran, C. D. Stiles, P. Leder, *ibid.* **35**, 603 (1983); J. C. Reed, J. C. Alpers, P. C. Nowell, R. G. Hoover, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3982 (1986); M. A. Shipp and E. L. Reinherz, *J. Immunol.* **139**, 2143 (1987).
22. J. B. Stern and K. A. Smith, *Science* **233**, 203 (1986).
23. G. Draetta, D. Beach, E. Moran, *Oncogene* **2**, 553 (1988).
24. J. Cheng *et al.*, *Blood* **75**, 730 (1990).
25. Y. Furukawa *et al.*, *Cancer Res.* **47**, 2589 (1987).
26. M. G. Lee and P. Nurse, *Nature* **327**, 31 (1987).
27. J. A. Swack *et al.*, *Mol. Immunol.* **26**, 1037 (1989).
28. J. Pines and T. Hunter, *Cell* **58**, 833 (1989).
29. E. L. Wickstrom *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1028 (1988); J. T. Holt, R. L. Redner, A. W. Nienhuis, *Mol. Cell. Biol.* **8**, 963 (1988).
30. We thank J. DeCaprio and B. Druker for critical review of the data and the manuscript; G. Draetta for the bacterial p13 overproducer; P. Nurse for pOB231; and C. Morimoto for the monoclonal antibody to CD6. Supported in part by PHS grants CA36167, CA47843, CA50767, and CA34183. Y.F. is supported in part by a fellowship from the Uehara Memorial Foundation and the Mochida Foundation for Medical and Pharmaceutical Research, and H.P.-W. by a Whitaker Health Sciences Fund Award. J.D.G. is a Scholar of the Leukemia Society of America.

8 May 1990; accepted 29 August 1990

Female Preference Predates the Evolution of the Sword in Swordtail Fish

ALEXANDRA L. BASOLO*

The study of female preferences and the evolution of male traits has until recently centered on genetic coevolutionary mechanisms. An alternative mechanism posits that a preference results from a preestablished bias in the female information-processing system arising from sources independent of sexual selection. Male traits then arise that are selected by this preexisting preference. The genus *Xiphophorus* consists of swordless platyfish and swordtails. Swordlessness is the primitive state. In this study, female platyfish, *X. maculatus*, were found to prefer conspecific males with artificial swords over those without swords, despite evidence that the common ancestor of platyfish and swordtails was swordless. These results suggest that the evolution of the sword in the swordtail clade was a consequence of selection arising from a preexisting bias.

SEXUAL SELECTION WAS FIRST PROPOSED by Darwin (*1*) as a mechanism to explain the evolution of elaborate traits in males that appear to decrease their survival. One type of sexual selection, female choice, involves a preference by females for traits in males. Until recently, models that stress the coevolution of a female preference and a male trait have dominated theoretical and empirical treatments (*2, 3*). Few data have been produced that support one of these models to the exclusion of another, and alternative, testable models have been proposed [see (*4*) for review]. A non-coevolutionary explanation for the evolution of a female preference and a male trait proposes that biases in the female sensory or cognitive system, or both, arise and increase to a high

---

University of Texas at Austin, TX 78712.

*Present address: Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

frequency, or fixation, for reasons independent of sexual selection (*5–9*). Subsequently, the evolution of male traits occurs when a newly arisen trait "exploits" preexisting biases in females. Thus, a preexisting bias in the female information-processing system, resulting from evolutionary processes independent of sexual selection, is present before the appearance of the male trait. Unlike models based on coevolutionary processes, a preexisting bias model does not require female preference to evolve because of sexual selection, nor does it require the coevolution of the preference and trait. However, after the male trait arises and is selected by the preexisting bias, other sources of selection may act in conjunction to further elaborate or modify the expression of the preference and the trait.

To demonstrate that preexisting biases have resulted in the evolution of a male trait, three criteria should be met. First, it must be established that in species in which the trait is present, there is female choice based on variation (either natural or artificial) in the trait (*10*). Second, it must be established that the absence of the male trait is the ancestral state within a clade. Third, it should be established that in a species having the primitive state, females prefer the male trait even though it was not generally present in the evolutionary history of the species. In this report, I address these criteria and present data that are consistent with a preexisting bias model for the evolution of the sword in the genus *Xiphophorus*.

Xiphophorus is a genus of small, freshwater fish in the live-bearing family Poeciliidae of Central America. The genus includes the platyfish, which are primarily swordless, and the swordtails, males of which typically have a sword, a colored extension of the lower margin of the caudal fin (*11, 12*). The sword does not develop until sexual maturity, at which time the rays at the base of the caudal fin begin to lengthen in males and acquire a border of black coloration. Swordlessness is considered to be the ancestral state for the genus (Fig. 1). I have shown that female green swordtails, *X. helleri*, prefer males with longer swords (*13*). The preexisting bias model predicts that male traits arise and are favored by preestablished preferences. If this is true, females in a closely related, swordless species such as *X. maculatus*, the southern platyfish (*14*), should also have a preference for a swordlike structure, even though the ancestral state of swordlessness is maintained. This hypothesis was tested through female choice tests to determine whether preference for a swordlike structure is a primitive state within the genus *Xiphophorus*.

Choice tests were conducted in an aquarium (45 cm by 41 cm by 87.5 cm) divided by two Plexiglas plates into three equal compartments (*13*). The middle compartment was divided into three sections: one center section and a section adjacent to each of the outer compartments. A test female was placed in an opaque tube suspended from above the center section, and a test male was placed in each of the outer compartments for a 10-min acclimation period. A test trial was initiated by raising the tube and releasing the female into the center section, beginning a 10-min observation period.

Table 1. Difference in time females spent with males of different sword status (SE in parentheses). "Yellow" represents males with artificial, 24-mm, surgically attached yellow plastic swords. "Transparent" represents males with 24-mm, surgically attached clear plastic swords, simulating the absence of a sword. Probabilities (*P*) are from Wilcoxon matched-pairs signed-rank test.

Body size (mm)	Mean time (s)		*n*	*P*
	Yellow	Transparent		
35	610 (25)	478 (26)	16	0.039
31	685 (39)	442 (37)	14	0.011
33	694 (67)	414 (64)	17	0.031
34	750 (41)	375 (44)	14	0.002
28	732 (39)	395 (28)	9	0.008
34	710 (54)	411 (53)	14	0.026

During the observation period, the behavior of the test subjects and the amount of time the female spent in each of the sections adjacent to the male compartments were recorded. After this period, the female was once more placed in the tube and the males were switched from one end compartment to the other (to control for side bias), and a second acclimation and observation period was conducted. During the choice tests, males and females actively courted one another. The time of the two observation periods was summed, and a Wilcoxon matched-pairs signed-rank test was used on the data set for each male-female combination to compare the difference in time spent with each of the males. Proximity, coupled with active courtship, was used as an indicator of female mating preferences; mating was not observed directly (*15*). Test subjects were either lab stock or progeny of wild-caught fish (*16*). Paired males were matched for body size (within 1-mm standard length) and body coloration.

I tested female preference for a sword structure in the swordless *X. maculatus* by surgically applying a plastic sword to platyfish males (*17*) and conducting female choice tests. Males were matched for body size and coloration. Paired males each received a 25-mm sword; one male received a yellow sword with a black border, the other a transparent sword (*18*). The transparent sword simulated the visual absence of a sword, while controlling for surgery effects and influences of the artificial sword on swimming behavior. Males were tested for female preference after a 3-day postoperative period. Males who underwent this surgery recovered and performed normal courtship behavior during female choice tests. Six pairs of males were tested, each with 9 to 16 females. In all six sets of tests, females preferred the male with the yellow sword to the male with the transparent sword (two-tailed binomial $P = 0.032$; Table 1).

An additional experiment, a sword reversal test, was conducted in which female preference was observed for a male with a yellow sword over a male with a transparent sword (Wilcoxon matched-pairs signed-rank test: $n = 14$, $P = 0.03$), and then the original swords were removed and replaced with the other type of sword. With this reversal in sword status, female preferences were reversed (Wilcoxon matched-pairs signed-rank test: $n = 14$, $P = 0.01$); females demonstrated a preference for the male with a yellow sword, although they had previously discriminated against him when he had a transparent sword. These data suggest that the females were basing their choice on sword presence and not other traits.

The absence of a male trait as the ancestral condition has been demonstrated in the frog genus *Physalaemus* (*7*). *Physalaemus pustulosus* females prefer male chucks at the low frequencies they detect best. In the closely related species, *P. coloradorum*, in which

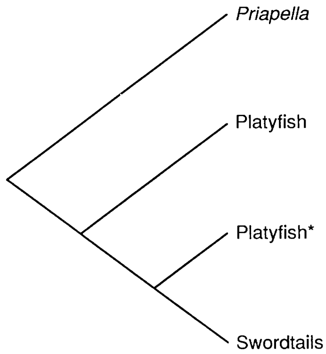

Fig. 1. Abbreviated phylogeny of the genus *Xiphophorus* (*11, 19*) (some additional species have been described, but phylogenetic analysis has not been published). The platyfish are generally unsworded, and most species of swordtails possess a sword (*11*). Swordlessness appears to be the primitive state, as the sister group, *Priapella*, does not possess a sword, and the sword, consisting of a colored extension at the ventral margin of the caudal fin, is a synapomorphy of the swordtail species (*19*). Morphological, electrophoretic, pigmentary, and morphometric characters were used to establish the phylogeny. Additionally, patterns of distribution were considered in some cases. (The clade to which the platyfish *X. maculatus* belongs is designated by an asterisk, *.)

males do not produce chucks, female neurophysiology also suggests that the inner ear is tuned to lower frequency calls. Therefore, female biases in the inner ear appear to have evolved before the production of low-frequency chucks in males. The present study with platyfish demonstrates that females exhibit a preexisting bias for a trait not generally present in the evolutionary history of the lineage. The three criteria necessary to demonstrate clearly that preexisting biases can result in the evolution of male traits have been met with this study: (i) there is variation for sword length in *X. helleri*, the green swordtail, and females prefer males with longer swords (*13*); (ii) the phylogeny for *Xiphophorus* suggests that the ancestral condition is swordlessness (*11, 19*) (Fig. 1); and (iii) in the swordless platyfish, *X. maculatus*, females demonstrate a preference for males with swords.

Obviously, only traits that lie within the detection limits of the female sensory and processing systems can be selected via female choice; thus, the potential range of sexually selected male traits is constrained. In preexisting bias models of sexual selection, the bases for the initial bias are numerous. A sword may increase the perceived body size of a male (*8*); thus, a preexisting preference for large males could, as a by-product, result in selection for the sword once the trait arises in a population. Similarly, a preexisting preference for rare, novel male types could initially select a swordlike structure; however, once an initially rare, novel trait becomes common, some other mechanism of preference or source of selection would be necessary to drive the trait to a high frequency or fixation. Therefore, preference for novelty per se cannot explain the evolution of the sword in swordtail fish. Female sensory or cognitive systems could be initially biased in response to environmental sources of selection. For example, a female may be adapted to recognize a moving shape in the environment because that moving shape resembles a favorite food, and females have developed a search image for it. This bias in the female detection system could subsequently bias the direction and nature of sexual selection if a mutation for males resembling this moving shape arises. In addition, new traits that increase conspicuousness could lead to selection for the trait. All of these possibilities share one common factor: the presence of the bias that results in a preference precedes the appearance of the male trait. In contrast, a coevolutionary mechanism requires the joint evolution of the trait and the preference.

A less parsimonious explanation for the preference of *X. maculatus* females for sworded males is that this preference is derived separately within the *X. maculatus* clade. Such a preference may be for novelty per se or some other characteristic. However, this interpretation of the results requires two independent evolutionary events: the evolution of a preference in the platyfish clade and the evolution of a sword preference in the swordtail clade. A preexisting bias hypothesis, in which a single evolutionary event accounts for a shared preference in platyfish and swordtails, is thus a more parsimonious explanation.

This work suggests that the common ancestor of the unsworded *X. maculatus* and the sworded *X. helleri* possessed a preference for a swordlike structure. Since phylogenetic hypotheses suggest that males of this common ancestor were unsworded, the preexisting bias resulting in a sword preference appears to have evolved before the sword itself; the sword thus may have subsequently arisen in the swordtail clade and been selected for by this preexisting bias. Models stressing coevolutionary processes cannot explain evolution of a female preference in the absence of a male trait, and, therefore, a preexisting bias model seems to best explain the evolution of the preference and the initial evolution of the sword. This conclusion does not preclude a role for coevolutionary processes. Once the sword has evolved, the initial bias and the trait could potentially be modified by other processes. However, the *Xiphophorus* system provides evidence for preexisting bias models of sexual selection; a role for other processes remains speculative.

REFERENCES AND NOTES

1. C. Darwin, *On the Origin of Species by Means of Natural Selection or the Preservation of Favored Races in the Struggle for Life* (John Murray, London, 1859); *The Descent of Man, and Selection in Relation to Sex* (John Murray, London, 1871).
2. A. Zahavi, *J. Theor. Biol* **53**, 205 (1975); R. Trivers, in *Sexual Selection and the Descent of Man, 1871–1971*, B. Campbell, Ed. (Aldine, Chicago, 1983), pp. 136–179; A. Kodric-Brown and J. H. Brown, *Am. Nat.* **124**, 309 (1984). Proponents of "good" genes suggest that one or a number of traits are used as an overall indicator of male fitness and that female preferences evolve under selection for females to mate with males who have genotypes favored by some source of natural selection.
3. R. A. Fisher, *The Genetical Theory of Natural Selection* (Dover, New York, 1930, 1958); R. Lande, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3721 (1981); P. O'Donald, in *Mate Choice*, P. Bateson, Ed. (Cambridge Univ. Press, Cambridge, 1983), pp. 53–66. Fisherian run-away selectionists stress the importance of linkage-disequilibrium between female preferences and sexually selected male traits: preferences evolve as a correlated response to male-trait evolution.
4. M. Kirkpatrick, *Ann Rev Ecol Syst.* **18**, 43 (1987); in *Sexual Selection: Testing the Alternatives*, J. W. Bradbury and M. B. Andersson, Eds. (Wiley, Chichester, United Kingdom, 1987), pp. 67–82.
5. G. W Barlow, in *How Animals Communicate*, T. A. Sebeok, Ed. (Indiana Univ. Press, Bloomington, 1977), p. 121; N. Burley, in *Avian Monogamy*, P. A. Gowaty and D. W. Mock, Eds. [*Ornithol. Monog. 37* (1985), pp. 22–44].
6. J. A. Endler and T. McLellan, *Annu. Rev. Ecol. Syst.* **19**, 395 (1988); J. A. Endler, in *Speciation and Its Consequences*, D. Otte and J. A. Endler, Eds. (Sinauer, Sunderland, MA, 1989), pp. 625–648.
7. M. J. Ryan *et al.*, *Nature* **343**, 66 (1990); M. J. Ryan and S. Rand, *Evolution* **44**, 305 (1990).
8. M. J. Ryan, *Ox. Surv. Evol. Biol* , in press.
9. Preexisting biases in the female sensory system have been termed both sensory drive (*6*) and sensory exploitation (*7, 8*); both of these terms are described as a bias in the direction of evolution that is set by the sensory system of a species. However, sensory drive applies to traits favored by natural selection, sexual selection, or a combination of both, and can affect the direction of evolution in the absence of sexual selection. Sensory exploitation applies specifically to sexually selected traits.
10. However, absence of a preference does not imply that a preference has never been present in the evolutionary history of the species and thus that female choice never played a role in the evolution of the trait.
11. M. Rauchenberger *et al.*, *Am Mus Novit. 2975* (1990).
12. A character generally shared by all swordtails is melanophore coloration on the ventral margin of the caudal fin. However, this coloration is absent in three northern swordtails (*11*).
13. A. L. Basolo, *Anim. Behav.* **40**, 332 (1990).
14. Wild *X maculatus* never develop an extension of the caudal fin, but they have been experimentally induced to produce a sword (*19*). Artificial selection has resulted in some fish with elongated upper and lower margins of the caudal fin, as can be seen in some *Poecilia* species. The males of two species of platyfish, *X xiphidium* and *X. andersi*, develop an unpigmented extension (1 to 5 mm) of the lower margin of the caudal fin (*11, 19*).
15. M. J. Ryan and W. E. Wagner, Jr., *Science* **236**, 595 (1987).
16. Test subjects either originated from lab stock populations maintained by K. D. Kallman, Osborn Genetics Laboratory, New York Aquarium, or were progeny from field-caught individuals from the Belize River, Belize, collected in 1986 and 1988. Lab stock males had either the recessive wild-type, "+," olivaceous body coloration and red irises, or had a speckled body (Sp) and red irises. Belize males were matched for any of a number of known color patterns.
17. Before application of the sword, the subject was sedated with MS-222. Surgical technique involved piercing the muscle tissue of the caudal peduncle with a 6-0 suture needle twice to secure the plastic sword. Three double reversed surgical knots were used to tie off the suture. The male's body was bathed in Novaqua and StressCoat during the 40 to 50 s that the procedure required, and Woundex was applied to the sutures after tie-off.
18. To exclude the possibility that the females were perceiving the transparent sword, a test was conducted in which preference for a male with a yellow sword was established, and then the transparent sword was cut back to a 10-mm nub that did not extend beyond the distal edge of the caudal fin. A second set of female choice tests was then conducted. In this test, preferences did not change. There was also no significant difference in the time spent with the male with the transparent sword or in the relative time spent with the male with the yellow sword as a result of this manipulation (A. L. Basolo, unpublished data).
19. D. E. Rosen, *Bull. Am. Mus. Nat. Hist.* **162**, 267 (1979).

20. Supported by NSF-Doctoral Dissertation Improvement grant BSR 8700944, a Theodore Roosevelt grant, the American Museum of Natural History, and a Raney Award, American Society of Ichthyologists and Herpetologists. I thank K. D. Kallman for advice and fish; the Ministry of Agriculture, Belize, for providing collecting permits; J. J. Bull, M. Kirkpatrick, and C. Pease for providing research space; J. Gardner for research assistance; and D. Barker, J. J. Bull, J. A. Endler, R. Gomulkiewicz, K. Kallman, M. Kirkpatrick, M. Ryan, W. Wagner, and three anonymous reviewers for constructive criticism of the experimental design and the manuscript.

27 March 1990; accepted 11 July 1990