Stabilizing selection on genomic divergence in a wild fish population

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Conservation programs use breeding protocols to increase genomic divergence (by mating genetically dissimilar individuals) in an attempt to circumvent population declines resulting from inbreeding depression. However, disruption of either beneficial gene complexes or local genetic adaptations can lead to outbreeding depression, and thus, there should be a reduction in fitness of individuals at either end of the genomic divergence continuum. Although such simultaneous inbreeding and outbreeding depression has been observed in plant populations, it rarely has been demonstrated in animal populations. Here, I use both genetic and phenotypic measures to show that there is stabilizing selection on genomic divergence in a wild population of bluegill sunfish (*Lepomis macrochirus*). I also show that breeding individuals that exercise mate choice produce offspring that are closer to the optimal level of genomic divergence than random mating alone would predict.

inbreeding | outbreeding | microsatellite | fitness | paternity

Genomic divergence is at the center of genetical studies of mating systems and conservation biology (1–3). Genomic divergence measures the genetic similarity or relatedness of an individual's parents. It has been shown that low genomic divergence resulting from close (incestuous) inbreeding can contribute to declines in wild populations and that mating systems have evolved to avoid incestuous breeding (4–7). Consequently, conservation programs have targeted methods to maximize genomic divergence in an attempt to circumvent inbreeding depression (2, 3). However, disruption of local adaptations or coadapted gene complexes can lead to outbreeding depression (8) and therefore there should be an optimal, intermediate level of divergence that maximizes fitness (1).

In nature, an optimum level of outbreeding has been demonstrated in several plant populations (9–11), but it rarely has been demonstrated in animal populations (12, 13). Arguably the best example comes from the plant *Ipomopsis aggregata*, where Waser and Price (10) showed that offspring produced from an intermediate outcrossing distance had higher lifetime fitness than those produced from either shorter or longer distances. In animals, an example comes from the Arabian oryx (*Oryx leucoryx*), which were hunted to extinction in the wild but successfully reintroduced into parts of their natural range from captive populations. Marshall and Spalton (12) used heterozygosity and mean d^2 (md^2) as measures of genomic divergence to show that individuals with low or high levels of genomic divergence had lower survivorship than individuals with intermediate levels.

The lack of evidence of simultaneous inbreeding and outbreeding depression (particularly in animal populations) may be due to difficulties in measuring genomic divergence; most examples involve some level of pedigree reconstruction and identification of migrants (e.g., ref. 13). When pedigree data are unavailable, multilocus heterozygosity commonly has been used to estimate genomic divergence, but this index is particularly suited to measuring low divergence (i.e., close inbreeding) and may be less suited for measuring high divergence. However, a relatively new measure called mean d^2 can provide information about genomic divergence across the entire continuum, and it should facilitate analysis of inbreeding and outbreeding depression.

Mean d^2 is based on the stepwise mutation model, which underlies the basic mutational dynamics of microsatellite loci, and it estimates the average divergence time between an individual's parents (14, 15). A large md^2 value implies that an individual's parents shared a more distant common ancestor ("relatively outbred"), and a small md^2 value implies that an individual's parents shared a more recent common ancestor ("relatively inbred"). Mean d^2 has been used in many studies to detect inbreeding and outbreeding depression. However, there has been considerable heterogeneity in results, and its ubiquitous application has been challenged (16–19).

A population genetic model has shown that heterozygosity is a better measure of inbreeding depression due to close inbreeding than md^2 (18). This result is supported by an empirical study on a wolf (*Canis lupus*) pedigree that showed that heterozygosity was better than md^2 at estimating the known inbreeding coefficient (17), and a recent metaanalysis, which showed that heterozygosity on average was better at detecting inbreeding depression (19). However, the latter analysis also showed that in several cases md^2 outperformed heterozygosity, and comparisons of studies in which both indices were used on the same data revealed no significant difference in performance. Furthermore, the population genetic model revealed that md^2 outperforms heterozygosity at detecting deep inbreeding (mixing of divergent lineages) when the product of effective population size and mutation rate at the marker loci is >1. Thus, for marker loci with mutation rates of 10^{-3} (e.g., many microsatellite loci), md^2 will outperform heterozygosity when the effective population exceeds 1,000 individuals. Such population sizes are not uncommon in many animal populations (particularly fish), and this may explain some of the heterogeneity conveyed by the metaanalysis.

Mean d^2 also may be better than heterozygosity at measuring high levels of genomic divergence. Using a simulation model, I found that md^2 provided a linear measure of the divergence time of lineages over a longer period than did heterozygosity (unpublished data). Furthermore, although multistep mutations (i.e., mutations of multiple repeats) marginally reduced the effectiveness of md^2, it still outperformed heterozygosity at detecting high levels of divergence. Thus, md^2 is reasonably impervious to deviations from a strict single-step mutation model.

In this article, I use md^2, heterozygosity, and a phenotypic measure of genetic quality to show that there is stabilizing selection on genomic divergence in a wild population of bluegill sunfish (*Lepomis macrochirus*). Furthermore, the data suggest that individuals capable of exercising mate choice select mates such that their offspring are closer to the optimal level of genomic divergence than predicted by random mating. These results may be common in fish populations.

Materials and Methods

Study Species. Bluegill are native to lakes and rivers of North America. Populations vary in size but typically are large, being

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composed of tens of thousands of individuals (21). Males are characterized by a discrete polymorphism in life histories termed "parental" and "cuckolder" (22). Parentals delay maturation and compete to construct nests in colonies during the breeding season. Nesting parentals court and spawn with females and provide sole parental care for the developing eggs and larvae in their nests. In contrast, cuckolders mature precociously and are parasitic, stealing fertilizations in the nests of parentals. On average, cuckolders fertilize only ≈20% of the eggs within a colony, but because of their higher survivorship to maturity they have similar fitness as that of parentals (22, 23). Over parts of their range, bluegill exist in sympatry with their sister species, pumpkinseed sunfish (*Lepomis gibbosus*), and occasionally hybridize. Bluegill also show nesting site fidelity. Therefore, breeding can occur between individuals that vary in their relatedness, ranging from close inbreeding to outbreeding with a different species (introgression).

Genetic Measurements. In the summer of 1996, a large net was used to enclose a bay in Lake Opinicon (Ontario, Canada), which contained a naturally breeding colony of bluegill sunfish (23, 24). At the end of the parental care period, all breeding adults and the larvae from each nest were collected. Genotypes at 11 microsatellite loci were determined for each adult and a sample of the offspring from each nest (described in refs. 23 and 24). Parentage models (25) were then used to calculate the proportion of offspring produced by each adult (the models can be downloaded from http://publish.uwo.ca/~bneff/links.htm). These models do not identify specific parent–offspring links, but instead adjust the proportion of offspring that are genetically compatible with a putative parent by the parent's exclusion probability. The estimates should be accurate because of the high exclusion probabilities (mean = 0.95, range = 0.81–1.00; see refs. 23 and 26).

Reproductive success was then calculated by weighting each nest by the brood size, which was estimated based on the dry weight of the larvae. Larvae were not directly counted because typically a nest contains tens of thousands of individuals, thus impeding accurate counting. Reproductive success was then standardized by using z scores within each life history (i.e., parental, cuckolder, and female) to remove life history-dependent reproductive success; for example, although cuckolders and parentals overall have similar fitness, they do not have similar reproductive success (22, 23). A z-score standardization is appropriate here because for all three life histories the reproductive success data did not differ from normality (Shapiro–Wilk test, $P > 0.08$ for each).

For each breeding adult and each sampled offspring, genomic divergence was calculated by using md^2 and heterozygosity. These measurements initially were compared among the three life histories by using nonparametric statistics because not all of the variables were normally distributed (Shapiro–Wilk test, $P < 0.05$). $\text{Log}_{10} + 1$ transformation restored normality in md^2 (Shapiro–Wilk test, $P > 0.19$ for each; unpublished data) and was used in all subsequent analyses. Arcsine square-root transformations did not restore normality in the heterozygosity data and thus nonparametric statistics always were used for these data.

Polynomial regression analysis was used to investigate the relationship between reproductive success and md^2. The procedure started with a higher-order polynomial than was expected to be significant and used a stepwise procedure to remove the term with the largest power until each remaining term in the model was significant. Theoretically, polynomial regressions allow curves of any shape to be fit to the data.

Mean d^2 also was calculated based on each combination of 10 loci (i.e., excluding one locus at a time) to determine whether the relationship revealed by the polynomial regression depended on any one locus. The proportion of these 11 regressions, each based on 10 loci, that were significant was compared to a null distribution generated from a randomization routine. The randomization routine generated a md^2 value for each parent based on only 10 loci. The omitted locus was randomly selected for each parent and therefore was not always the same locus. The routine was repeated for a total of 100 data sets from which regression analysis was used to generate the null distribution of P values; i.e., the expected proportion of significant results based only on 10 loci.

Spearman's correlation was used to investigate the relationship between heterozygosity and reproductive success, as well as the residuals of reproductive success from the polynomial regression with md^2.

Phenotypic Measurements. Three phenotypic measurements were taken from each adult. First, Fulton's condition factor was calculated as weight divided by the cube of total body length. This index correlates with mobile lipid density in fish and specifically in bluegill (B.D.N. and L. M. Cargnelli, unpublished data). Second, parasite load was quantified based on counts of five parasite types known to actively feed on bluegill comprising *Dactylogyrus* sp., *Ergasilus caeruleus*, *Proteocephalus* sp., *Spinitectus* sp., and *Leptorhynchoides* sp. (27). To account for size-specific metabolic costs of each parasite type, parasite number was adjusted for their relative body weights. Third, fluctuating asymmetry was calculated from 11 bilateral traits consisting of the number of pectoral fin rays, length of longest pectoral fin ray, number of pelvic fin rays, length of longest pelvic fin ray, number of teeth (left and right side of upper palette), number of gill rakers (four sets), dry weight of black opercular flap extension, and dry weight of otoliths. For each trait, fluctuating asymmetry was calculated as the difference in the natural logarithm of the left and right values, and these values were standardized by using z scores. The absolute value of the z scores were then averaged across the 11 traits to provide a single composite index. The z score weights each trait equally in the overall index (28). Polynomial regression analysis (for md^2) and Spearman's correlation (for heterozygosity) were used to investigate the relationships between the phenotypic measurements and genomic divergence. Before analysis, each phenotypic measure was standardized within the life histories by using z scores to remove any life history-dependent variation.

Mate Choice. To examine mate choice for genomic divergence, offspring were first partitioned into two categories: parental or cuckolder. Offspring were assigned to the parental group when they shared at least one allele at all 11 loci with the nest-tending parental (nest take-overs have never been observed in bluegill); otherwise, offspring were assumed to be sired by a cuckolder. Based on the average exclusion probability, ≈5% of cuckolder offspring would have been incorrectly assigned to the nest-tending parental because of chance matches at all 11 loci. Conversely, genotype scoring error and mutations would result in some parental offspring being incorrectly assigned to cuckolders. Based on a sample of 171 individuals genotyped twice at all 11 loci, scoring error was estimated to be <0.4%. Assuming a per locus mutation rate of 0.1%, the probability that there is a mutation at any of the 11 loci is 1.1%. Thus, ≈1.5% (= 0.4 + 1.1) of cuckolder offspring would have been incorrectly assigned to parentals. Although these incorrect assignments will add "noise" to the analysis, they are unlikely to explain any relationship uncovered in the analysis.

The average md^2 of parental and cuckolder offspring were then calculated. For comparison, null distributions of md^2 that assumed random mating were separately calculated for parental and cuckolder males. These distributions were generated by randomly selecting a male (either parental or cuckolder) and a female from the breeding population and generating one off-

Table 1. Summary of the measurements of genomic divergence for the three bluegill life histories

	Mean d^2		Heterozygosity	
Life history	Mean	Range	Mean	Range
Parental (n = 39)	28.0	3.5–78.5	0.57	0.27–0.91
Cuckolder (n = 58)	25.1	5.3–88.6	0.58	0.27–1.0
Female (n = 45)	27.1	3.0–58.6	0.60	0.37–1.0

spring based on Mendelian inheritance patterns. A total of 1,397 and 402 offspring were generated for parental and cuckolder males, respectively. These values are the number of offspring actually assigned to parental and cuckolder males from the colony. The average md^2 of the offspring were then calculated, and the process was repeated 999 times to generate the null distribution. The 95% confidence interval (C.I.) was determined by sorting the 1,000 values and selecting the 25th and 975th values, and the distribution was used to determine the probability of observing the actual md^2 for parental or cuckolder offspring under the assumption of random mating.

Results

Bluegill parentals, cuckolders, and females had similar levels of genomic divergence (Table 1) as measured by md^2 (Kruskal–Wallis test, $\chi^2 = 1.09$, d.f. = 2, $P = 0.58$) and heterozygosity (Kruskal–Wallis test, $\chi^2 = 0.55$, d.f. = 2, $P = 0.76$). In all three groups, the two measures of genomic divergence were correlated (parentals, $r_s = 0.39$, $P = 0.016$, $n = 39$; cuckolders, $r_s = 0.42$, $P = 0.001$, $n = 58$; females, $r_s = 0.38$, $P = 0.011$, $n = 45$).

Examining all individuals, the polynomial regression analysis of reproductive success and md^2 revealed that a quadratic regression was the highest-order polynomial in which all terms were significant; individuals with intermediate levels of genomic divergence had higher reproductive success as compared to those that had either low or high levels ($r^2 = 0.11$, $P < 0.001$, $n = 142$; Fig. 1*a*). Furthermore, by dividing individuals into three categories, evenly spaced based on md^2, analysis confirmed that intermediate individuals had significantly higher reproductive success than individuals with either low or high levels of genomic divergence (ANOVA, $F_{2,139} = 4.9$, $P = 0.01$). Similar quadratic relationships were found when parentals, cuckolders, and females were analyzed separately. However, only the relationships for parentals and cuckolders were significant ($P < 0.02$ for both), possibly because females had less variance in genomic divergence, with fewer females that had high levels of md^2 (see Table 1).

Analysis of all permutations excluding one of the 11 loci revealed one nonsignificant regression (Table 2). However, the randomization analysis revealed that 23% of the regressions based on any 10 loci were nonsignificant (at $\alpha = 0.05$). Thus, it was expected that, because of reduced power, 2.5 ($= 0.23 \times 11$) of the 11 comparisons in Table 2 should be nonsignificant. As such, the one nonsignificant result may represent a type II error.

There was a positive correlation between heterozygosity and reproductive success ($r_s = 0.151$, $P = 0.073$, $n = 142$) and heterozygosity and the residual reproductive success not explained by md^2 ($r_s = 0.175$, $P = 0.038$, $n = 142$). This latter result suggests that the effect of heterozygosity was additional to the effect captured by md^2.

There was no apparent relationship between any of the three phenotypic measures and heterozygosity or any of these measures and md^2 except for fluctuating asymmetry. Individuals with either low or high values of md^2 had higher levels of fluctuating asymmetry [quadratic regression, $r^2 = 0.05$, $P = 0.028$, $n = 134$ (complete asymmetry values could not be obtained for 8 individuals); Fig. 1*b*]. A Bonferroni correction was not used here

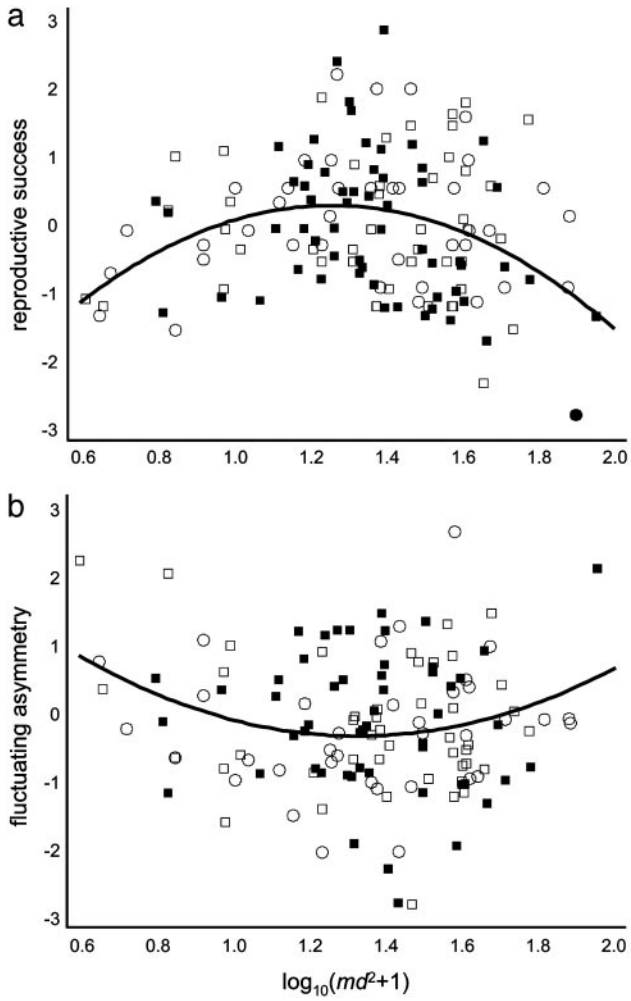

Fig. 1. Stabilizing selection on genomic divergence in bluegill sunfish. (*a*) The relationship between $\log_{10}(md^2 + 1)$ and reproductive success (*rs*). The line represents the quadratic equation: $rs = 8.09 \log_{10}(md^2 + 1) - 3.21 \log_{10}(md^2 + 1)^2 - 4.82$; each coefficient was significant ($P < 0.001$). The filled circle (lower right) denotes a single bluegill–pumpkinseed hybrid (F_1) parental male that nested in the colony. Exclusion of this male did not affect the significance of the result. The *rs* was standardized within each group by using *z* scores because the average reproductive success differs between the groups (see text). (*b*) The relationship between $\log_{10}(md^2 + 1)$ and fluctuating asymmetry (*fa*). The line represents the quadratic equation: $fa = -5.75 \log_{10}(md^2 + 1) + 2.16 \log_{10}(md^2 + 1)^2 + 3.60$; each coefficient was significant ($P < 0.01$). Open circles denote parental males, filled squares denote cuckolder males, and open squares denote females.

because the six comparisons were not independent as assumed by the correction; heterozygosity and md^2 were correlated, as were some of the phenotypic measures (B.D.N. and L. M. Cargnelli, unpublished data).

Offspring of parentals had significantly lower values of md^2 ($\log_{10} + 1$ transformed) than expected under random mating [observed, 1.340; expected under random mating, 1.405, 95% confidence interval (C.I.) = 1.390–1.420; $P < 0.001$]. These offspring had md^2 values that were closer to the optimum (= 1.260) that maximizes reproductive success as predicted from the quadratic regression (see Fig. 1*a*). In contrast, offspring of cuckolders had significantly higher values of md^2 than expected under random mating (observed, 1.414; expected under random mating, 1.377, 95% C.I. = 1.348–1.406; $P = 0.006$), and these offspring had values that were significantly larger than those of parental offspring ($t_{1797} = 4.23$, $P < 0.001$) and further from the

Table 2. Quadratic regression analysis of reproductive success (*rs*) and md^2 for all permutations excluding one of the 11 loci

Omitted locus	r^2	Coefficients*		
		C_1	C_2	C_3
Lma102	0.05*	2.41*	2.56**	2.10*
Lma87	0.11**	6.66***	2.73***	3.79***
Lma21	0.08**	6.45***	2.54***	3.88**
Lma117	0.10**	5.70***	2.34***	3.24***
Lma122	0.09***	6.06***	2.39***	3.64***
Lma121	0.11**	6.55***	2.62***	3.83***
Lma120	0.08**	5.73**	2.31***	3.31**
Lma116	0.04*	3.27**	1.40**	1.76*
Lma20	0.03^{ns}	0.23^{ns}	0.29^{ns}	0.21^{ns}
Lma124	0.10***	5.60***	2.28***	3.18***
Lma113	0.07**	4.84**	2.02**	2.68**

The r^2 represents the explained variation of the model, and the coefficients (C_1 or C_2) and constant (C_3) are listed for each equation: $rs = C_1 \log_{10}(md^2 + 1) - C_2 \log_{10}(md^2 + 1)^2 - C_3$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, $P > 0.05$.

optimum. Across all offspring, the md^2 values were not significantly different from those of the parents [offspring, mean = 1.362 ± 0.312 (SD), range = 0.16–2.58; parents, mean = 1.356 ± 0.278, range = 0.60–1.95; $t_{1939} = 0.213$, $P = 0.83$].

Discussion

This study demonstrates stabilizing selection on genomic divergence in a wild animal population based on both a genetic measure (md^2) and a phenotypic measure (fluctuating asymmetry). Mean d^2 apparently is better than heterozygosity at measuring deep inbreeding when the product of effective population size and mutation rate of the marker loci is >1 (18). Assuming the microsatellites used in this study have a mutation rate of 10^{-3}, a commonly reported value (29), the effective population size would need to be >1,000 individuals for the product to exceed 1. Detailed census and tracking data of breeding individuals in Lake Opinicon indicate that the breeding population is likely at least an order of magnitude more than 1,000 individuals (22, 30). Although variance in reproductive success among individuals (23) will reduce the effective population from the number of breeders, it likely exceeds 1,000 individuals. Thus, in this population md^2 should be better than heterozygosity at detecting deep inbreeding. Mean d^2 also may be better at detecting high levels of genomic divergence due to outbreeding (unpublished data), and this may explain why there was a significant quadratic relationship between md^2 and reproductive success, indicating depression due to both deep inbreeding and outbreeding.

Heterozygosity, on the other hand, is better than md^2 at detecting close (incestuous) inbreeding (18). Here, a positive relationship between heterozygosity and reproductive success was found, and this relationship captured a greater amount of variation when reproductive success was first controlled for the effect of md^2. This finding indicates that there also is close inbreeding depression. Thus, collectively the data suggest that individuals that have high genomic divergence (relative to the population mean) or low genomic divergence due to either close or deep inbreeding are less fit.

Additional support for inbreeding and outbreeding depression in bluegill comes from the analysis of fluctuating asymmetry. Vrijenhoek and Lerman (31) proposed fluctuating asymmetry as a measure of developmental stability relating to genomic divergence: individuals of intermediate genomic divergence are expected to have the greatest developmental stability and display the lowest levels of asymmetry (also see refs. 32 and 33). Although several studies have found no relationship between fluctuating asymmetry and fitness, metaanalysis has revealed that there is a negative relationship (34, 35). I found that individuals with either low or high genomic divergence as measured by md^2 were more asymmetrical than individuals of intermediate levels of divergence. This result suggests that individuals of intermediate genomic divergence have higher genetic quality and thereby are better able to canalize the development of their bilateral traits.

The variation in reproductive success and fluctuating asymmetry accounted for by either measure of genomic divergence was small. This is expected because fitness-related traits typically display high residual variation and low heritability (36, 37). Indeed, metaanalysis has shown that correlations between phenotypic variation and either md^2 or heterozygosity predominately are weak, although those for life history traits are stronger than those for morphological traits (19).

It does not appear as though the relationship between reproductive success and md^2 depends on any one locus. When Lma20 was excluded from the analysis the quadratic relationship was not significant, and when either Lma102 or Lma116 was excluded the explained variance was reduced by about half, although the relationship remained significant (Table 2). However, the randomization analysis indicated that this variance was not unexpected because of the reduced power of using only 10 loci. Nevertheless, it cannot be ruled out that some of the loci are linked to fitness loci that actually drive the relationship.

Parental males and females appear to select mates such that their offspring have intermediate levels of genomic divergence. Only parentals actively court females; cuckolders are opportunistic spawners that dart in and out of the nests of parentals. Consequently, mate choice predominately operates between parentals and females, and these individuals appear to select mates such that their offspring are closer to the optimum level of genomic divergence that maximizes reproductive success. The mechanism of this choice might relate to odor cues derived from the major histocompatibility complex (MHC). MHC has been implicated in mate choice through olfactory assessment (38–41), and parental male bluegill can assess relatedness based on an individual's odor (42, 43). Conversely, cuckolder offspring were further from the optimum level of genomic divergence than even random mating would predict (likely a consequence of mate choice between parental males and females). The mating behavior of cuckolders will contribute to the maintenance of variation in genomic divergence within the population, as will any recognition errors during mate choice (44).

Variation at the MHC may provide a mechanism for the observed inbreeding depression in bluegill. Either close or deep inbreeding could lead to reduced variation at MHC genes. These genes are responsible for immunological responses to infection, and diversity is believed to provide greater immunity (38). Indeed, a study on a songbird has shown that females pick extra-pair mates (i.e., genetic mates outside of their social pair bond) based on MHC dissimilarity (40). Bluegill with low genomic divergence also may have lower diversity at the MHC, leading to a compromised immune system.

The mechanism of outbreeding in bluegill is unclear. In the Arabian oryx, there is a segregating chromosomal polymorphism in the reintroduced population, suggesting that divergent lineages have been combined (12). Outbreeding depression in bluegill similarly may relate to disruption of coevolved gene complexes. For example, one individual was a hybrid (likely F_1) between a bluegill and a pumpkinseed sunfish. The hybrid had one of the highest values of genomic divergence and also was relatively unsuccessful at reproducing (although the hybrid successfully nested, he was heavily cuckolded). Analysis of the hybrid's gonads suggested that sperm production was compromised (unpublished data). Male bluegill have special-

ized sperm that have evolved in response to sperm competition imposed by the alternative male reproductive life histories (45, 46). Thus, introgression of genomes could disrupt gene complexes such as those involved in sperm production and may be one mechanism that contributes to the observed outbreeding depression.

The mechanism of outbreeding in bluegill also may relate to the disruption of local adaptations. Bluegill are characterized by a foraging polymorphism with some individuals developing into a "limnetic" morph that specializes in feeding on zooplankton in open waters, whereas other individuals develop into a "littoral" morph that specializes in feeding on insect larvae in shallow waters (47). Although the morphs feed in different habitats, they breed in the same habitat at the same time. Thus, assuming the morphs have a genetic basis, disassortative mating would produce individuals that are less effective at capturing prey in either habitat and could reduce their fitness (20).

In conclusion, the bluegill population studied here appears to demonstrate both inbreeding and outbreeding depression. Such phenomenon may be common in fish populations because many are characterized by philopatry, yet some level of migration between locally adapted populations can occur. The data from bluegill as well as other fish (e.g., ref. 8) suggest that fish conservation programs exclusively targeting methods to increase genomic divergence could be overlooking outbreeding depression. These programs might benefit from targeting an optimal, intermediate level of genomic divergence, which could be accomplished by incorporating natural mate choice into their protocols.

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1. Thornhill, N. W., ed. (1993) *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (Univ. of Chicago Press, Chicago).
2. Keller, L. F. & Waller, D. M. (2002) *Trends Ecol. Evol.* **17,** 230–241.
3. Wang, S. Z., Hard, J. J. & Utter, F. (2002) *Rev. Fish Biol. Fish.* **11,** 301–319.
4. Keller, L. F., Arcese, P., Smith, J. N. M., Hochachka, W. M. & Stearns, S. C. (1994) *Nature* **372,** 356–357.
5. Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. (1998) *Nature* **392,** 491–494.
6. Madsen, T., Shine, R., Olsson, M. & Wittzell, H. (1999) *Nature* **402,** 34–35.
7. Tregenza, T. & Wedell, N. (2002) *Nature* **402,** 71–73.
8. Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C. & Quinn, T. P. (2000) *Science* **290,** 516–518.
9. Price, M. V. & Waser, N. M. (1979) *Nature* **277,** 294–297.
10. Waser, N. M. & Price, M. V. (1989) *Evolution (Lawrence, Kans.)* **43,** 1097–1109.
11. Waser, N. M. (1993) in *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives*, ed. Thornhill, N. W. (Univ. of Chicago Press, Chicago), pp. 173–199.
12. Marshall, T. C. & Spalton, J. A. (2000) *Anim. Conserv.* **3,** 241–248.
13. Marr, A. B., Keller, L. F. & Arcese P. (2002) *Evolution (Lawrence, Kans.)* **56,** 131–142.
14. Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. (1998) *Proc. R. Soc. London Ser. B* **265,** 489–495.
15. Coltman, D. W., Bowen, W. D. & Wright, J. M. (1998) *Proc. R. Soc. London Ser. B* **265,** 803–809.
16. Goudet, J. & Keller, L. (2002) *Trends Ecol. Evol.* **17,** 201–202.
17. Hedrick, P., Fredrickson, R. & Ellegren, H. (2001) *Evolution (Lawrence, Kans.)* **55,** 1256–1260.
18. Tsitrone, A., Roussett, F. & David, P. (2001) *Genetics* **159,** 1845–1859.
19. Coltman, D. W. & Slate, J. (2003) *Evolution (Lawrence, Kans.)* **57,** 971–983.
20. Ehlinger, T. J. (1990) *Ecology* **71,** 886–896.
21. Lee, D. S., Gilbert, C. R., Hocutt, C. H., Jenkins, R. E., McAllister, D. E. & Stauffer, J. R., Jr. (1980) *Atlas of North American Freshwater Fishes* (State Mus. of Nat. Hist., Raleigh, NC).
22. Gross, M. R. (1982) *Z. Tierpsychol.* **60,** 1–26.
23. Neff, B. D. (2001) *J. Hered.* **92,** 111–119.
24. Neff, B. D. & Gross, M. R. (2001) *Proc. R. Soc. London Ser. B* **268,** 1559–1565.
25. Neff, B. D., Repka, J. & Gross, M. R. (2000) *Mol. Ecol.* **9,** 515–528.
26. Neff, B. D., Repka, J. & Gross, M. R. (2000) *Mol. Ecol.* **9,** 529–540.
27. Muzzall, P. M. & Peebles, C. R. (1998) *J. Helminthol. Soc. Wash.* **65,** 201–204.
28. Leung, B., Forbes, M. R. & Houle, D. (2000) *Am. Nat.* **155,** 101–115.
29. Jarne, P. & Lagoda, J. L. (1996) *Trends Ecol. Evol.* **11,** 424–429.
30. Cargenelli, L. M. & Gross, M. R. (1996) *Can. J. Fish. Aquat. Sci.* **53,** 360–367.
31. Vrijenhoek, R. C. & Lerman, S. (1982) *Evolution (Lawrence, Kans.)* **36,** 768–776.
32. Leary, R. F., Allendorf, F. W. & Knudsen, K. L. (1983) *Nature* **301,** 71–72.
33. Møller, A. P. & Swaddle, J. P. (1997) *Asymmetry, Developmental Stability, and Evolution* (Oxford Univ. Press, New York).
34. Møller, A. P. & Thornhill, R. (1998) *Am. Nat.* **151,** 174–192.
35. Møller, A. P. (1998) *(1999) Am. Nat.* **153,** 556–560.
36. Gustafsson, L. (1986) *Am. Nat.* **128,** 761–764.
37. Houle, D. (1992) *Genetics* **130,** 195–204.
38. Edwards, S. V. & Hedrick, P. W. (1998) *Trends Ecol. Evol.* **13,** 305–311.
39. Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. (2001) *Proc. R. Soc. London Ser. B* **268,** 1279–1285.
40. Freeman-Gallant, C. R., Meguerdichian, M., Wheelwright, N. T. & Sollecito, S. V. (2003) *Mol. Ecol.* **12,** 3077–3083.
41. Aeschlimann, P. B., Häberli, M. A., Reusch, T. B. H., Boehm, T. & Milinski, M. (2003) *Behav. Ecol. Sociobiol.* **54,** 119–126.
42. Neff, B. D. & Sherman, P. W. (2003) *Anim. Cogn.* **6,** 87–92.
43. Neff, B. D. (2003) *Nature* **416,** 716–719.
44. Randerson, J. P., Jiggins, F. M. & Hurst, L. D. (2000) *Proc. R. Soc. London Ser. B* **267,** 867–874.
45. Fu, P., Neff, B. D. & Gross, M. R. (2001) *Proc. R. Soc. London Ser. B* **268,** 1105–1112.
46. Neff, B. D., Fu, P. & Gross, M. R. (2003) *Behav. Ecol.* **14,** 634–641.
47. Ehlinger, T. J. & Wilson, D. S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1878–1882.