Increased Infectious Disease Susceptibility Resulting from Outbreeding Depression

TONY L. GOLDBERG,*†‡§ EMILY C. GRANT,† KATE R. INENDINO,† TODD W. KASSLER,** JULIE E. CLAUSSEN,‡ AND DAVID P. PHILIPP†‡§

*Department of Veterinary Pathobiology, University of Illinois at Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL 61802, U.S.A., email tlgoldbe@uiuc.edu

†Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1102 South Goodwin Avenue, Urbana, IL 61801, U.S.A.

‡Illinois Natural History Survey, Center for Aquatic Ecology, 607 East Peabody Drive Champaign, IL 61820, U.S.A.

§Program in Ecology and Evolutionary Biology, University of Illinois at Urbana-Champaign, 286 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, U.S.A.

**Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, WA 98501-1091, U.S.A.

Abstract: *The mechanisms by which outbreeding depression leads to reduced fitness are poorly understood. We considered the hypothesis that outbreeding can depress fitness by increasing the susceptibility of hybrid individuals and populations to infectious disease. Competitive breeding trials in experimental ponds indicated that outbred largemouth bass (*Micropterus salmoides*) crossed from two geographically and genetically distinct populations suffered a reduction in fitness of approximately 14% relative to parental stocks. We measured the comparative susceptibility of these same outbred stocks to a novel viral pathogen, largemouth bass virus. Following experimental inoculation, F2 generation hybrids suffered mortality at a rate 3.6 times higher than either F1 generation hybrids or wild-type parental fish. Analysis of viral loads indicated that viral replication was more rapid in F2 fish than in F1 hybrids or wild-type parental fish. We attribute these results to the disruption of coadapted gene complexes in the immune systems of outbred fish in the F2 generation. Increased susceptibility to infectious disease may be an important but underappreciated mechanism by which outbreeding reduces the fitness of individuals and populations and by which novel infectious diseases emerge in populations of hybrid organisms.*

Key Words: hybrids, largemouth bass virus, reduced fitness

Incremento de la Susceptibilidad a Enfermedades Infecciosas Debido a Depresión Exogámica

Resumen: *Los mecanismos por los que la depresión exogámica conduce a la reducción de adaptabilidad son poco conocidos. Consideramos la hipótesis de que la exogamia puede deprimir la adaptabilidad al aumentar la susceptibilidad de individuos y poblaciones híbridas a enfermedades infecciosas. Pruebas de reproducción competitiva en estanques experimentales indicaron que la adaptabilidad de lobinas (*Micropterus salmoides*) exogamas cruzadas de dos poblaciones geográfica y genéticamente distintas tuvo una reducción de aproximadamente 14% en relación con los troncos parentales. Medimos la susceptibilidad comparativa de estas mismas poblaciones exogamas a un patógeno viral novedoso, virus de la lobina Micropterus salmoides. Después de la inoculación experimental los híbridos de la generación F2 tuvieron una tasa de mortalidad 3.6 veces mayor que la generación F1 de híbridos o que peces silvestres. El análisis de cargas virales indicó que la replicación viral fue más rápida en peces F2 que en híbridos F1 o en peces silvestres. Atribuimos estos resultados a la alteración de complejos de genes coadaptados en los sistemas inmunes de peces exogamos en la generación F2. El incremento en la susceptibilidad a enfermedades infecciosas puede ser un mecanismo importante, pero*

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subestimado, mediante el cual la exogamia reduce la adaptabilidad de individuos y poblaciones y mediante el cual emergen nuevas enfermedades infecciosas en poblaciones de organismos híbridos.

Palabras Clave: híbridos, reducción de adaptabilidad, virus de la lobina *Micropterus salmoides*

Introduction

Outbreeding depression is defined as a reduction in fitness of hybrid individuals and populations caused by the disruption of locally or intrinsically coadapted gene complexes (Templeton 1986; Frankham 1999). Although the phenomenon of outbreeding depression has been recognized since the time of Dobzhansky (1948), the mechanisms by which it occurs in nature are poorly understood. In particular, it is unknown whether excessively outbred individuals suffer reduced fitness inherently or only when exposed to external stressors that render them poorly suited to specific environments.

Increased disease susceptibility is one possible mechanism through which outbreeding depression might operate. The complexity of immune systems and their associated gene complexes should make them particularly sensitive to disruption following hybridization events. To address this hypothesis, we studied the responses of largemouth bass (*Micropterus salmoides*) to a newly discovered viral pathogen of North American warmwater fishes, largemouth bass virus (LMBV; family Iridoviridae).

Largemouth bass virus is an emerging pathogen in the genus *Ranavirus* (Plumb et al. 1996; Mao et al. 1999). First described in 1996, it has been associated with fish kills in the southeastern and central United States (Plumb et al. 1996; Goldberg 2002; Grizzle et al. 2002). The virus can infect multiple fish species but has been associated with epidemic mortality only in the largemouth bass and the Florida bass (*Micropterus floridanus;* Goldberg 2002). Largemouth bass virus has caused conspicuous kills in economically important bass populations and persists in affected water bodies (Hanson et al. 2001; Grizzle & Brunner 2003). The virus may be a recently introduced, emerging pathogen, or it may have been present, undetected, for years. In either case, it provides an excellent model for investigating disease susceptibility in centrarchid fishes. The LMBV causes rapid mortality in experimentally infected largemouth bass, and techniques for its propagation and quantification in vitro and in vivo are well established (Piaskoski et al. 1999; Plumb & Zilberg 1999; Goldberg et al. 2003).

We conducted an experiment in which we exposed outbred largemouth bass to LMBV under controlled conditions. We began by selecting two populations of bass to serve as parental stocks for the breeding of hybrids. We chose populations from the upper midwestern United States that displayed only a small degree of genetic differentiation. We then crossed fish from these populations to create outbred hybrids (F1 and F2 generations). We compared the fitness of these hybrid stocks to that of the parental stocks from which they were derived in a series of competitive breeding trials. Finally, we exposed the parental stocks and the hybrid outcrosses to live virus and compared their sensitivities to infection.

We predicted that outbred fish would suffer both reduced fitness and reduced tolerance to viral infection. We further predicted that these effects would be most pronounced in F2 generation fish. Our predictions are based on the idea that coadapted gene complexes can become disrupted as a result of meiosis and recombination during gametogenesis in F1 generation hybrids, such that the depressive effects of outbreeding should express themselves most strongly as functional deficits in the offspring of F1 parents. If substantiated, these predictions would not only implicate increased infectious disease susceptibility as an important mechanism for outbreeding depression, but would also raise new concerns about the health and viability of natural populations when such populations contain both hybrid organisms and harmful pathogens.

Methods

Genetic Characterization of Parent Populations

In 1995 we collected wild adult largemouth bass from two geographically separate watersheds in the upper midwestern United States: the Kaskaskia River in Illinois (KR; Mississippi River basin) and Big Cedar Lake in Wisconsin (BC; Great Lakes basin). The fish were transported to outdoor holding ponds at the Illinois Natural History Survey (Champaign).

We characterized these two populations genetically and compared them with a geographically representative subset of largemouth bass populations from the upper midwestern United States. Data consisted of allozyme allele frequencies (sample size of 30 individuals per population) from 26 loci, seven of which were polymorphic (*sAAT-B**, *sMDH-B**, *LDH-C**, *G2D-1**, *CK-C**, *PGM-1**, and *sSOD**). These loci and the methods used to characterize them are fully described elsewhere (Philipp et al. 1983, 2002). We used the computer program Biosys (version 1.7; Swofford & Selander 1981) and Nei's (1978) unbiased genetic distance method to calculate genetic distances

between pairs of populations. We represented the genetic relationships among the populations as a neighbor-joining phylogram (Saitou & Nei 1987). We calculated the degree of genetic differentiation between the two parent populations as Wright's (1978) F_{ST}.

Production of Study Organisms

We obtained fin clips from each adult bass collected from both study populations (BC and KR) and used starch gel protein electrophoresis to determine individual genotypes at the *sMDH-B** locus (Philipp et al. 2002). For the BC population, we used only adults homozygous for the *sMDH-B*^{1} allele for further breeding. For the KR population, we used only adults homozygous for the *sMDH-B*^{2} for further breeding.

In 1996 we placed these wild-captured, genotyped adults in ponds (five pairs in each of two ponds) to produce offspring of the following crosses through natural spawning: P1: BC female × BC male, genotype = *sMDH-B*^{1}*B*^{1}; P1: KR female × KR male, genotype = *sMDH-B*^{2}*B*^{2}; F1: BC female × KR male, genotype = *sMDH-B*^{1}*B*^{2}; F1: KR female × BC male, genotype = *sMDH-B*^{1}*B*^{2}.

We collected equal numbers of age-0 offspring of each cross from ponds, gave them cross-specific fin clips for identification, and reared them together until they were mature adults. In 2002 we paired fish (age 6) and allowed them to spawn naturally in ponds to create offspring of three levels of outbreeding: nonoutbred parental (P1) fish, first-generation (F1) interpopulation hybrids, and second-generation (F2) interpopulation hybrids. The F2 hybrids were offspring of each of the two reciprocal crosses (male × female and female × male) that we created in the F1 generation.

Comparative Fitness of Outbred Largemouth Bass

To assess the relative fitness of the outbred and nonoutbred largemouth bass, we conducted competitive breeding trials in experimental spawning ponds at the Illinois Natural History Survey (Champaign). Details of rearing and sampling methods were similar to those given elsewhere (Philipp & Claussen 1995; Philipp et al. 2002). Briefly, we introduced equal numbers of adult fish (20 males and 20 females from the 1996 year class) from each of the two stocks being compared into experimental breeding ponds in which we allowed them to spawn naturally (Table 1). We determined the relative fitness of each lineage by sampling age-0 offspring in September and measuring the frequencies of the *sMDH-B*^{1} and *sMDH-B*^{2} alleles in those fish. We quantified relative fitness by dividing the observed frequency of each allele by its expected frequency under the assumption of equal reproductive input from the two competing stocks (Philipp & Claussen 1995; Philipp et al. 2002).

Table 1. Fitness of parental stocks of largemouth bass compared with outbred F1 interstock crosses in experimental reproduction competition ponds.

Experiment	*Competing stocks*[a]	n[b]	$\%W_r$ *(range)*[c]
A	P1 vs. P1 (KR vs. BC)	474	102.0 (97.0–105.0)
B	P1 vs. F1 (KR vs. KR × BC)	456	85.9 (81.6–90.4)*
C	P1 vs. F1 (KR vs. BC × KR)	486	86.0 (82.4–90.8)*

[a]*Abbreviations: KR, Kaskaskia River; BC, Big Cedar Lake. Genotypes of competing stocks were: KR female × KR male (*sMDH-B^{2}B^2*) versus BC female × BC male (*sMDH-B^{1}B^1*) for experiment A; KR female × KR male (*sMDH-B^{2}B^2*) versus BC female × KR male (*sMDH-B^{1}B^2*) for experiment B; and KR female × KR male (*sMDH-B^{2}B^2*) versus KR female × BC male (*sMDH-B^{1}B^2*) for experiment C.*

[b]*Number of fish genotyped per experiment. Each experiment consisted of four independent replicates (two ponds in 2001 and two ponds in 2002).*

[c]*Relative fitness (W_r) was measured as the frequency of allozyme alleles observed in the offspring generation divided by the expected frequency assuming equal reproductive input from the two competing stocks. Ranges indicate relative fitnesses observed across the four replicate ponds in each experiment. Asterisks indicate comparisons that are significantly different from null expectation, as determined by one-sample* t *tests (3 df).*

Virus and Cell Culture

The virus used for experimental inoculation of juvenile bass was a sixth cell culture passage of the original-type isolate collected from Santee Cooper Reservoir in South Carolina (Plumb et al. 1996). To generate this virus, we inoculated confluent monolayers of fathead minnow (*Pimephales promelas*) cells with fifth cell culture passage LMBV at a multiplicity of infection of 0.1. We performed inoculations in 150-cm^2 tissue culture flasks containing 25 mL of Eagle's minimum essential medium with Hank's salts, 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, and 50 μg/mL gentamycin sulfate, incubated at 30° C for 5 days or until we observed maximum cytopathic effect. We harvested cell culture supernatant and filtered it through a 0.45-μm filter to remove cellular debris. We then titered this virus by inoculating serial dilutions onto replicate cell culture monolayers in 96 well plates. We calculated titers ($TCID_{50}$/mL) according to the method of Karber (1931). We used filtered cell culture supernatant from an uninfected flask of cells for sham injections of control fish.

Experimental Challenge of Bass with Live Virus

The fish used for viral challenge were 13–14 months of age with a mean length of 108 mm (±0.7 SE) and a mean weight of 13 g (±0.4 SE). Clinical testing of a representative sample of fish, including brood stock, showed that the populations were negative for LMBV.

We collected two fish from each parental stock (P1) and two fish from each F1 and F2 generation hybrid cross from holding ponds and placed them together in each of 12 replicate 76-L aquaria, for a total of 144 fish (12 fish per tank, 48 fish from each level of outbreeding). Unique patterns of fin clips allowed us to identify fish from each cross unambiguously. We kept the tanks in a closed environmental chamber under constant ambient temperature (25°C), humidity (60%), and photoperiod (12 hours light/12 hours dark). We allowed fish to equilibrate to indoor conditions for 1 week before starting the experiment. We fed fish mosquito larvae once daily (approximately 0.8 g/fish).

We did not feed fish for 24 hours prior to inoculation with LMBV. On day 0 of the experiment, we netted fish from tanks and placed them in temporary holding containers. We captured each fish by hand and exposed it to 10^6 $TCID_{50}$ of virus via intraperitoneal injection of 0.1 mL of Hank's balanced salt solution (HBSS) containing 10^7 $TCID_{50}$/mL (Plumb & Zilberg 1999). We injected virus with a tuberculin syringe and 27 G, 1/2-in. needle inserted into the coelomic cavity of each fish along the ventral midline. We returned fish to their respective tanks immediately following injection. Fish from 10 tanks (120 fish total; 40 fish each of P1, F1, and F2) received live virus. Fish from two tanks (24 total; 8 fish each of P1, F1, and F2) received 0.1 mL of virus-free cell culture supernatant and served as sham-injected controls.

We monitored tanks at 24-hour intervals for 3 weeks after exposure. During this observation period, we removed all dead fish. We euthanized moribund fish (fish that were listless and displayed loss of equilibrium) in an emulsion of clove oil (Peake 1998; Taylor & Roberts 1999). For each fish, we recorded the date of removal, tank number, and genetic cross. At the end of the observation period, we euthanized all surviving fish.

Processing of Fish

We processed fish for gross pathology and quantification of viral load immediately after removing them from tanks. We individually weighed and dissected each fish with flame-sterilized forceps and scissors. We quantified body condition as Fulton's index ($[\text{weight}/\text{length}^3] \times 10^5$), a common measure used for comparing fish condition within and among populations (Bolger & Connolly 1989). We recorded external and internal gross lesions. We then removed the viscera of each fish (including the gastrointestinal tract, reproductive organs, swim bladder, kidney, spleen, liver, heart, and mesentery), weighed them, and diluted them in 50 volumes of HBSS containing 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, and 50 μg/mL gentamycin sulfate in a sterile plastic bag. We placed each bag in a Stomacher 80 Biomaster laboratory blender (Seward, Norfolk, United Kingdom) for 60 seconds at normal speed to homogenize the viscera. We stored the homogenates at −80° C for DNA extraction and quantification of viral load. We quantified viral load with a real-time quantitative polymerase chain reaction (PCR) technique and expressed it as the number of viral genomes per gram of fish tissue, according to previously published methods (Goldberg et al. 2003).

Statistical Analysis

We conducted statistical analyses with SAS (version 8.2, SAS Institute, Cary, North Carolina). We analyzed survival data with Cox proportional hazard regression. We included in our regression models both fixed effects representing differences between the generations of outbreeding (P1, F1, and F2) and random effects representing random differences in mortality between tanks within treatments. We analyzed viral load data with multiple linear regression in which we constructed orthogonal contrast variables to represent differences in the state of fish at the time of death (found dead, found moribund, or survived to the end of the experiment) and differences between the generations of outbreeding (P1, F1, and F2). We included random tank-effect variables as described above. We considered associations to be statistically significant at the $\alpha = 0.05$ level.

Results

Analyses of allozyme allele frequencies revealed that the two parental stocks of bass used in this study were typical of populations found in the upper midwestern United States (Fig. 1; Philipp et al. 1983). The F_{ST} between the parental stocks, averaged across all seven polymorphic loci, was 0.05, which was near the middle of the range of F_{ST} values between pairs of the populations (Fig. 1, range of 0 to 0.13). The two stocks were much more closely related to other largemouth bass populations than to this species' closest relative, the Florida bass (*M. floridanus*). We interpret these data as indicating that KR and BC are two closely related populations of the same species that have undergone only a small degree of genetic differentiation.

The competitive breeding experiments revealed that the two P1 stocks (KR and BC) had equal fitness (Table 1, experiment A; $\chi^2 = 0.624, p = 0.43$). The F1 hybrid individuals from both reciprocal crosses, however, suffered a reduction in fitness of approximately 14% compared with the nonoutbred KR stock (Table 1, experiments B and C; $\chi^2 = 2.63$, $p = 0.05$ and $\chi^2 = 3.36$, $p = 0.03$, respectively).

Upon experimental exposure to LMBV, the two reciprocal crosses within each generation of outbreeding (i.e., within the P1, F1, or F2 generations) experienced mortality at statistically indistinguishable rates; we therefore

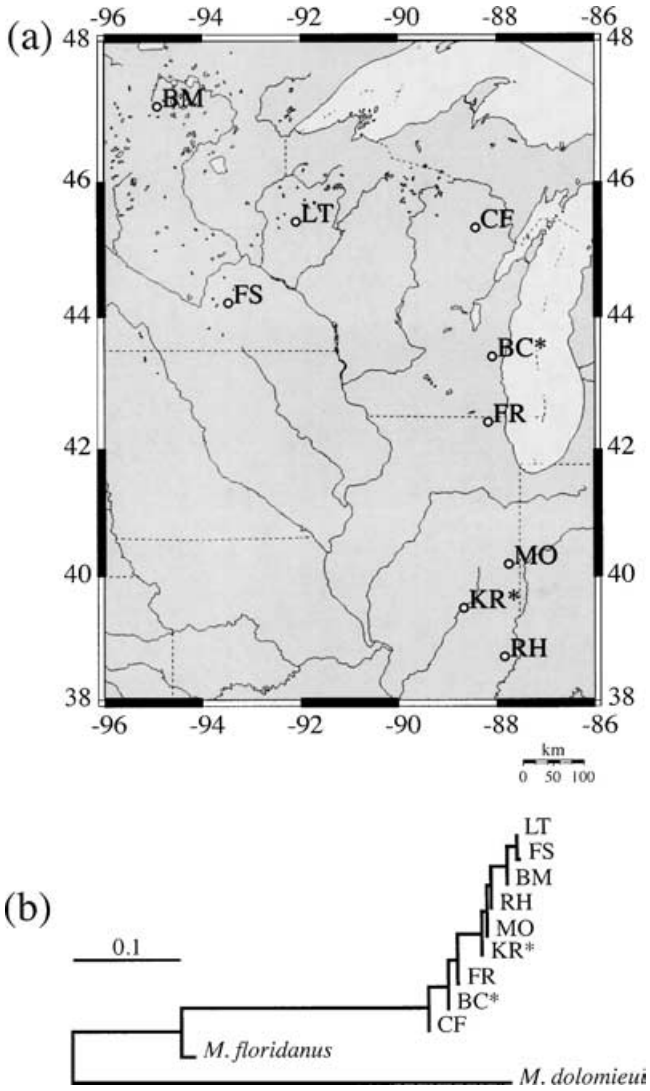

*Figure 1. Geographic and genetic relationships among largemouth bass populations from the upper midwestern United States. (a) Map of sampling locations, with abbreviations referring to water bodies: LT, Lipsett; FS, Frances; BM, Big Mantrap; RH, Red Hills; MO, Mingo; KR, Kaskaskia River; FR, Fox River; BC, Big Cedar; CF, Caldron Falls. The map was created with Generic Mapping Tools (Wessel & Smith 1991) through Online Map Creation (http://www.aquarius.geomar.de/). (b) Neighbor-joining phylogram constructed from allozyme allele frequency data of largemouth bass (30 individuals per population genotyped at seven polymorphic loci), with the Florida bass (*M. floridanus*) and the smallmouth bass (*M. dolomieui*) included as outgroups. Asterisks indicate the populations from which fish were sampled for the outbreeding study.*

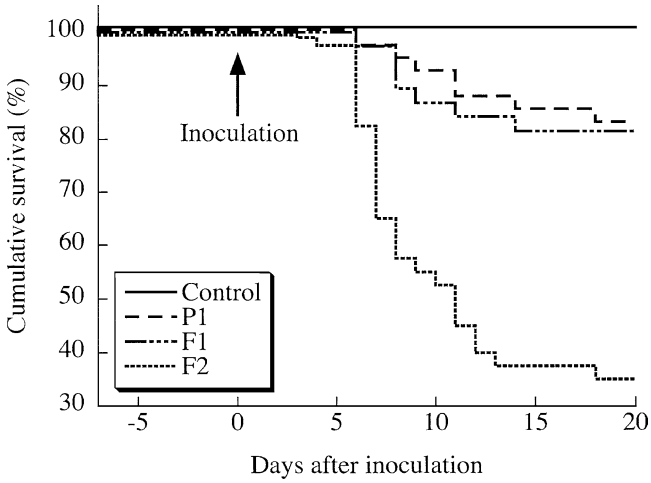

Figure 2. Cumulative survival of largemouth bass exposed to largemouth bass virus. No mortality was observed in sham-injected control fish of any level of outbreeding (P1 parental stocks, F1 generation outcrosses, or F2 generation outcrosses), so results from all control fish were combined and represented as a single line.

combined data from the two reciprocal crosses for further analysis. Differences in mortality between P1 and F1 fish were not statistically significant, with 83.3% and 81.6% survival 2 weeks after exposure, respectively (hazard ratio = 1.08; $\chi^2 = 0.08$; $p < 0.77$; Fig. 2). The F2 fish, however, suffered markedly increased mortality compared with P1 and F1 fish, with only 35% survival 2 weeks after exposure (hazard ratio = 3.55; $\chi^2 = 29.87$; $p < 0.0001$; Fig. 2). No sham-injected control fish died during the observation period.

No statistically significant differences existed in viral load among P1, F1, or F2 fish (Table 2). Fish that were moribund or died during the observation period had higher viral loads than fish that survived to the end of the experiment ($t = 3.27$; $p < 0.001$). Viral load was negatively associated with body condition of the fish at the time of death ($t = -3.47$; $p < 0.001$). Neither the presence of external ($t = 0.02$; $p = 0.98$) nor internal ($t = -0.30$; $p = 0.76$) gross lesions significantly predicted viral load. Viral loads of sham-injected control fish were below minimum detection limits and were statistically indistinguishable from negative control real-time quantitative PCRs.

Discussion

Effects of Outbreeding on Fitness and Disease Susceptibility

Our results indicate that hybrid largemouth bass suffered marked outbreeding depression. The genetic contribution of F1 generation hybrid bass to each year class

Table 2. Viral loads of juvenile largemouth bass exposed to equal doses of largemouth bass virus.

	State of fish			
	found dead or moribund		*survived to end of experiment*	
Outcross[a]	n	*viral load*[b]	n	*viral load*[b]
P1 parental	7	9.78 ± 0.15	33	6.46 ± 0.35
F1 outbred	9	9.63 ± 0.18	31	6.43 ± 0.34
F2 outbred	26	9.98 ± 0.06	14	6.79 ± 0.44

[a] *Results for reciprocal outcrosses did not differ and were combined for analysis.*
[b] *Viral loads are expressed as* $\log_{10}$ *viral genomes per gram of tissue* ± *standard error.*

produced was reduced by 14% relative to nonoutbred fish, as shown by competitive breeding trials in experimental ponds (Table 1). The mechanisms causing this reduction are not clear, but could involve the reduced fecundity and/or mating success of F1 parents, the reduced survival of F2 offspring, or some combination of these two effects. Either effect would be consistent with the hypothesis that outbreeding disrupts intrinsically coadapted gene complexes.

A clearly depressive effect of outbreeding was evident in the response of fish to infection with LMBV. The F2 generation outbred bass were dramatically more sensitive to viral infection than were F1 generation hybrids or the parental stocks from which those hybrids were derived (Table 2). Specifically, infected F2 fish died more than 3.5 times faster than either F1 generation fish or parental stocks. This outcome was surprisingly strong considering the small degree of genetic differentiation between the two original parental populations ($F_{ST} = 0.05$).

The observation that viral load did not differ among fish across the different levels of outbreeding, coupled with the observation that F2 fish died more rapidly than did either P1 or F1 fish, indicates a difference among stocks in the in vivo kinetics of viral replication. The virus must have replicated to a threshold lethal level more quickly in F2 fish than in F1 or P1 fish. We estimate this threshold level as the mean viral load of all fish that died during the observation period, or $10^{9.9\pm0.4}$ viral genomes/g of tissue.

Viral loads of fish that survived to the end of the experiment were markedly lower than those of fish that died during the observation period. By the end of the 3-week observation period, mortality rates appeared to have reached a "plateau" in all three levels of outbreeding (Fig. 2). It is currently unclear why some fish within treatments were able to reduce viral loads and remain apparently healthy while others succumbed quickly. Viral load was negatively associated with body condition at the time of death. This result may indicate either that fish in better condition at the outset of the experiment were better able to limit viral replication or that fish with high viral loads lost body condition disproportionately during the course of the experiment. Furthermore, the fact that neither internal nor external gross lesions were associated with viral load confirms that gross pathology is a poor indicator of infection in this system (Plumb et al. 1996; Zilberg et al. 2000; Grizzle & Brunner 2003).

Mechanisms of Outbreeding Depression

The observed decrease in survival of hybrids challenged with LMBV is consistent with the hypothesis that outbreeding depression can occur as a result of the disruption of coadapted gene complexes (Templeton 1986). The appearance of this effect in the F2 generation, but not in the F1 generation, implies the disruption of such complexes as a result of meiosis and recombination between chromosomes inherited from parents of different lineages in F1 fish. We consider it likely that the gene complexes involved function in the highly coordinated immune system and that their disruption reduces the efficiency of the immune response. Such effects have been suggested as explanations for increased helminth loads in naturally hybridizing populations of mice (*Mus musculus* and *Mus domesticus*; Sage et al. 1986) and for increased susceptibility to myxosporean parasites in naturally hybridizing rainbow trout (*Oncorynchus mykiss*; Currens et al. 1997).

Because the agent involved in this study was a virus, and thus an obligate intracellular pathogen, we speculate that reductions in cell-mediated immunity, rather than humoral immunity, are responsible for the effect observed. We predict that the negative consequences of outbreeding will persist into future generations (F3 and beyond) until the genes responsible are eventually linked again into functional complexes as a result of natural selection for immune response efficiency. In mixed populations of hybrid and nonhybrid individuals, selection may disfavor hybrid genotypes, a process that can reestablish populations of parental genotypes quickly when selection is strong (Templeton 1986).

We conclude that hybridization between individuals from closely related populations can generate offspring with increased susceptibility to infectious diseases. The magnitude of such effects may be directly proportional to the degree of genetic divergence between the source populations. For example, Templeton et al. (1976) showed that the number of surviving offspring of outbred female *Drosophila mercatorum* decreased approximately linearly with increasing degrees of hybridity, a finding consistent with the disruption of intrinsically coadapted gene complexes (Templeton 1986). Vrijenhoek and Lerman (1982), however, argue in favor of a threshold effect, in which hybrid offspring become rapidly inviable beyond a critical degree of outbreeding. Because we examined hybrids between only a single pair of populations, an accurate description of the shape of the relationship

between the degree of outbreeding and infectious disease susceptibility must await further research.

Reductions in fitness of outbred individuals have been documented across a variety of taxa in natural and experimental settings (e.g., Gharrett & Smoker 1991; Aspi 2000; Fenster & Galloway 2000; Neff 2004). Such fitness reductions have been attributed to aberrant mating behavior, physiological performance deficits, lowered or inefficiently apportioned parental investment, and lowered offspring survival rates. Nevertheless, the underlying mechanisms by which these phenotypic responses become manifest in hybrid individuals are poorly understood. Increased susceptibility to pathogens that subsequently cause both overt and subtle phenotypic effects may be an important, but heretofore underappreciated, mechanism by which outbreeding depression operates. The failure to document outbreeding depression in some studies (e.g., Holtsford 1996; Luijten et al. 2002; Pflugshaupt et al. 2002; Bieri & Kawecki 2003) may not reflect the lack of a genetic effect, but rather the lack of its expression in environments containing few pathogens.

Such effects apparently do not depend on prior exposure of hosts to a specific pathogen or on a history of host-pathogen coevolution that would create immune-system gene complexes adapted to a particular infectious agent. Our evidence indicates that the fish populations we used had not previously been exposed to LMBV. If so, outbreeding may lead to a generalized reduction in the functional efficiency of the immune response that will reduce the resistance of organisms to a broad range of insults, infectious and otherwise.

Relevance to Conservation

Our results are particularly relevant to emerging infectious diseases of wildlife. Translocation of wildlife by humans is an important anthropogenic factor implicated in the spread and emergence of novel infectious wildlife diseases (e.g., Viggers et al. 1993; Woodford & Rossiter 1993; Cunningham 1996). Outbreeding depression is also a significant risk associated with moving locally adapted organisms into new habitats in which they can interbreed with resident organisms (Moritz 1999; Cross 2000). The results of our study indicate that these two effects may be synergistic. Outbreeding may decrease the resistance of wildlife populations to infectious agents exactly where and when such agents are introduced through the translocation of infected hosts. This could, in turn, facilitate the emergence of novel diseases and could precipitate epidemics.

For example, interspecific crosses between largemouth bass and Florida bass suffer approximately 50% reductions in fitness (Philipp et al. 2002). We speculate that the large-scale translocation of the Florida bass outside of its native range and across much of the southeastern United States, where the largemouth bass also occurs (Philipp et al. 2002), may be a factor contributing to the epidemic of LMBV-associated fish kills that has been documented in U.S. waters since 1995 (Goldberg 2002). To the extent that hybridization exacerbates the population-level effects of infectious disease, the interaction between outbreeding and infectious disease susceptibility will have conservation-related impacts. These impacts may extend beyond the introgressed populations themselves and may affect other populations or species to which hybrids transmit pathogens.

Overall, our results suggest that increased infectious disease susceptibility resulting from outbreeding depression should be considered a risk to the conservation of populations of hybrid individuals and their ecosystems, even when the degree of genetic divergence between the introgressed source populations is small (as was the case in this study). Maximum disease resistance in populations of conservation interest may be achieved most efficiently not by maximizing outbreeding, but rather by restricting outbreeding to levels that maintain genetic diversity but simultaneously prevent the disruption of coadapted gene complexes. Captive breeding programs, artificial stocking programs, and programs focused on translocating or reintroducing threatened species for purposes of augmenting diminished local populations should be reevaluated in this light.

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