Repeat Swimming Performance and Its Implications for Inferring the Relative Fitness of Asexual Hybrid Dace (Pisces: *Phoxinus*) and Their Sexually Reproducing Parental Species

Jonathan A. Mee*
Colin J. Brauner
Eric B. Taylor
Department of Zoology, University of British Columbia,
6270 University Boulevard, Vancouver, British Columbia
V6T 1Z4, Canada

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ABSTRACT

While theories explaining the evolution and maintenance of sex are abundant, empirical data on the costs and benefits of asexual relative to sexual reproduction are less common. Asexually reproducing vertebrates, while few, provide a rare opportunity to measure differences in fitness between asexual and sexual species. All known asexually reproducing vertebrates are of hybrid origin, and hybrid disadvantage (i.e., reduced hybrid fitness) is thought to facilitate long-term coexistence between asexual and sexual species. We used repeat swimming performance as a proxy for fitness to compare the fitness of asexual hybrid dace (Pisces: *Phoxinus*) and their sexually reproducing parental species, finescale dace (*Phoxinus neogaeus*) and northern redbelly dace (*Phoxinus eos*). We tested the prediction that, given the widespread coexistence of these hybrid and parental dace, the parental species should show equivalent and perhaps superior repeat performance relative to hybrids. A repeat constant acceleration test ($U_{\max}$) was conducted at both acclimation temperature (16°C) and at an elevated temperature (25°C) to simulate the combined influence of a repeat swim and acute temperature change that fish might experience in the wild. The asexual hybrids performed more poorly than at least one of the parental species. There was a negative effect of temperature on repeat swimming performance in all fish, and the repeat performance of hybrids was more severely affected by temperature than that of finescale dace. No difference in the effect of temperature on repeat performance was detected between hybrids and northern redbelly dace. These results suggest that hybrids suffer physiological costs relative to the parentals or at least that the hybrids do not gain advantage from hybrid vigor, which probably contributes to the coexistence of asexual and sexual species in this system.

Introduction

Asexual reproduction is rare among vertebrates; depending on how one defines an asexual "species," there are only 30–90 known vertebrate examples (Dawley and Bogart 1989; Avise 2008). In contrast with the low number of asexual species, the theoretical advantages of asexual reproduction are numerous and have been of interest for well over a century (Darwin 1862; Felsenstein 1974; Williams 1975; Barash 1976; Maynard Smith 1978; Charlesworth 1980; Hastings 1999). The most commonly cited and perhaps the most intuitive and important advantages of asexual reproduction include the fact that asexuals pass all their genes to their offspring, whereas sexuals pass only half their genes on to their offspring (Charlesworth 1980), and asexual populations have double the potential growth rate of sexual populations (Lively 1996; Jokela et al. 1997). Based on these advantages, it is perhaps surprising that asexual reproduction among vertebrates is not more common. The observation that asexuality is rare combined with the theoretical expectation that it should be more common has motivated the development of many hypotheses to explain how the advantages of asexual reproduction are balanced or outweighed by advantages inherent to sexual reproduction (Kondrashov 1993; Agrawal 2006). Although evolutionary biology is not lacking in theories for why sex is advantageous and common, a thorough understanding of the reasons why sexual reproduction is common, particularly among vertebrates, can only be achieved through empirical study of closely related asexual and sexual species.

All known asexual vertebrates have originated from hybridization (Dawley and Bogart 1989), which may have an important influence on their fitness relative to sexual vertebrates. Hybrids may suffer disadvantages because of incompatibilities between the parental species' genomes, but hybrids may also gain advantages by the creation of novel beneficial genetic variants resulting in hybrid vigor. Hybrid vigor might be expected to arise spontaneously among asexual vertebrates because of the high degree of genetic heterozygosity characteristic of interspecific hybrids (leading, perhaps, to a "general purpose genotype"), or it might be expected to result from interclonal selection leading to the persistence of only the most fit hybrid genotypes (Lynch 1984). There is, in fact, evidence for hybrid vigor in some asexual vertebrate systems (Hotz et al. 1999;

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* Corresponding author; e-mail: mee@zoology.ubc.ca.

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Kearney et al. 2005). In contrast, several theoretical models have shown that the long-term coexistence of asexual vertebrates with their sexual parental species is facilitated if asexuals do not gain a fitness benefit from hybrid vigor and especially if the asexuals suffer a fitness loss because of hybrid disadvantage (Moore 1976; Hellriegel and Reyer 2000; Mee and Otto 2010). Hybrid disadvantage has been detected in several asexual vertebrate systems (Wetherington et al. 1987; Lively et al. 1990; Moritz et al. 1991; Weeks 1995; Cullum 1997; Vorburger 2001; Mee and Rowe 2006). It is important to note that these contrasting empirical results (i.e., some asexual vertebrates showing hybrid vigor and some showing hybrid disadvantage) may be due to variation in the age of these hybrid asexual lineages. Additional data on the ages of asexual vertebrate lineages and on components of fitness of asexual vertebrates and their parental species are required for a thorough analysis of the advantages and disadvantages at play in the maintenance of sex among vertebrates.

Two species of dace (Pisces: Cyprinidae), northern redbelly dace (*Phoxinus eos* Cope, 1861) and finescale dace (*Phoxinus neogaeus* Cope, 1867), have produced asexually reproducing hybrids that co-occur across much of the parental species' northern North American ranges. The same clonal hybrid lineages have probably been coexisting with parental dace populations across their range ever since the last glacial retreat (Angers and Schlosser 2007). Like many asexual vertebrates, these hybrids are sperm-dependent asexuals and reproduce via gynogenesis—sperm from a parental species is required for egg development but not for its genetic constitution (Goddard et al. 1998). Many hybrid dace populations coexist with only one of the two parental species, but several lakes have been shown to contain both parentals and the hybrid. Even in the lakes with both parental species, there is no evidence that hybridization has occurred since the last glacial retreat. Most hybrid dace coexist with northern redbelly dace, but a few populations have been found in Alberta and northeastern British Columbia coexisting with only finescale dace (J. A. Mee, personal observation). These fishes are an excellent model system in which to evaluate relative fitness as a factor facilitating the coexistence of asexual and sexual forms because while there is some evidence that the hybrids are ecological generalists relative to the parentals, there is a substantial amount of ecological similarity between hybrid and parental dace (Schlosser et al. 1998; Doeringsfeld et al. 2004). Ecological differentiation can, on its own, promote coexistence between sexual and sperm-dependent asexual species (e.g., Vrijenhoek 1979, 1994). Hence, ecological similarity suggests that the asexuals and sexuals must, to some degree, be in direct competition for the same resources, and any fitness-related trait would likely have an important influence on coexistence.

Piscivorous fishes and birds commonly prey on dace, and the ability of a dace to evade a predator by engaging in high-intensity swimming multiple times in succession likely has important fitness consequences. An evasive response that included sustained high-intensity swimming away from a predator was important for the survival of fathead minnows (*Pimephales promelas*; Webb 1982), a cyprinid species that co-occurs with *Phoxinus* dace across much of its range. Acute exposure to temperatures above optimal values, especially when approaching upper lethal temperatures, imposes an additional stressor that may reduce swimming performance whether the swim test is aerobic or anaerobic in nature (Beamish 1978; Randall and Brauner 1991). The ability of a fish to swim fast enough to evade a predator is likely affected by incomplete recovery from a previous evasive swim as well as by temperature, but the influence of an acute temperature change on repeat swimming performance has not previously been evaluated.

The functional morphology and physiology of swimming performance is a critical aspect of the life of aquatic animals. In fact, tests of swimming ability have been used for decades to test hypotheses concerning the evolution of adaptations of migratory and stream-dwelling fishes (e.g., Brett 1964; Taylor and McPhail 1985, 1986; Taylor and Foote 1991; Brauner et al. 1994; Nelson et al. 2003). Further, tests of repeat swimming performance (i.e., when an animal is tested twice in rapid succession) have been used to study fish health and the metabolic processes involved in recovery from exhaustion (Jain et al. 1998; Jain and Farrell 2003).

In this study, we used a test of repeat swimming performance to compare the swimming ability, as a proxy for fitness, of asexual hybrid dace with that of its sexually reproducing parental species. We addressed the effects of multiple stresses, namely repeat swimming and increased temperature, on swimming ability by conducting the repeat swimming performance test at both a laboratory acclimation temperature and at an elevated temperature. We hypothesized that, given the widespread long-term coexistence of these asexual hybrid and sexually reproducing parental dace, the asexuals should have hybrid disadvantage (i.e., reduced fitness relative to the parentals) or at least should lack hybrid vigor.

Material and Methods

Study Populations

Females from four lakes with populations of hybrid and parental dace were brought into the lab in the summer of 2007. These lakes included two from Alberta and two from Ontario. The Alberta populations were from Alford Lake (N52°03′50″, W115°05′32″) and Strubel Lake (N52°12′09″, W115°0′4″) near the town of Caroline. Alford Lake contains populations of finescale and hybrid dace (Alford Lake contains no northern redbelly dace), while Strubel Lake contains populations of northern redbelly and hybrid dace (Strubel lake contains no finescale dace). The Ontario populations were from Pondweed Lake (N42°27′48″, W78°27′30″) and the outlet creek from Sunday Lake (N45°35′14″, W78°22′12″) in Algonquin Provincial Park. Neither Ontario lake contained a finescale dace population—both contained only northern redbelly and hybrid dace populations. Fish from each lake were kept in 95-L tanks (all the fish from a given lake in a single tank) with no flow, and these tanks were housed in an environmental chamber with a 12L : 12D cycle and kept at 16°C. Fish were fed ad lib. with a mixture of frozen bloodworms and frozen daphnia once daily. One of several colors of elastomer dye was injected subcutaneously at each of four dorsal landmarks (relative to the dorsal fin: front left, front right, back left, back right) to give each fish a unique tag and track individuals throughout the protocol described below. Several fish died within the first month after being brought to the environmental chamber, but there were no mortalities in the several months leading up to the experiment, and no mortalities occurred during the experiment or as the result of tagging.

Repeat Swimming Performance Test Protocol

Beginning in September 2008, every fish was subjected to a repeat constant acceleration swimming performance test (U_{max}) at acclimation temperature (16°C) and at an elevated temperature (25°C). These temperatures were chosen because fish tended to remain healthy and active over the long term at 16°C, and 25°C was the highest temperature at which dace acclimated to 16°C were consistently able to perform a constant acceleration test during pilot experiments. The constant acceleration test (U_{max}) has been shown to be equivalent to the more commonly employed critical swimming speed test (U_{crit}) in juvenile pink salmon (*Oncorhynchus gorbuscha*; Nendick et al. 2009), but it tends to overestimate U_{crit} by up to 57% in adult rainbow trout (Farrell 2008). The U_{max} test is a much shorter duration test than the U_{crit} test and likely more closely approximates the type of swim that a lake-dwelling cyprinid might perform when escaping a predator under natural conditions. The U_{max} test likely consists of both an anaerobic and aerobic component, but it is possible that this test may have been predominantly fueled by glycolytic white muscle activity (Farrell 2008).

These repeat U_{max} tests were intended to estimate the simultaneous effects of a repeat swim and elevated temperature (i.e., the effect of multiple stressors) on the maximum swimming speed, U_{max}, attainable by each fish. Before being tested, fish were deprived of food for between 40 and 48 h in order to standardize any potential influence of digestion or feeding status on swimming performance or behavioral motivation. Three submersible water heaters, immersed in the tank around the 10-L Loligo swim tunnel (Loligo Systems APS), were used to raise the temperature in the swim tunnel before the tests at 25°C (the heaters were left on for the duration of the 25°C tests). For the tests at 16°C, the heaters were turned off but left in the tank, and either the apparatus was left for several hours to return to the ambient temperature in the environmental chamber or cold water was added to hasten the return to ambient temperature. At the beginning of each test, after the water in the swim tunnel had stabilized at the desired temperature, a single fish was transferred to the swim tunnel and left to habituate for 45 min at a water velocity of 7.5 cm/s. Fish were encouraged to swim by gentle prodding with a probe as necessary during the habituation period. Following habituation, an initial measure of swimming performance, U_{max1}, was performed by increasing the flow rate by 2.5 cm/s at 30 s intervals until the fish fatigued, which was defined as that point when the fish collapsed (perpendicular to the direction of flow) against the back grid of the swim tunnel for the third time. A second measure of swim performance, U_{max2}, was then performed immediately following U_{max1} (i.e., no recovery period following U_{max1}) using the same procedure as U_{max1} starting at 10 cm/s. Values of U_{max} were calculated following Brett (1964):

$$U_{max} = u + t/30\ \mathrm{s} \times 2.5\ \mathrm{cm/s},$$

where u is the highest speed at which the fish swam for the entire 30-s interval (cm/s) and t is the time the fish swam before becoming exhausted in the final interval (s). The mean durations (not including habituation) of each stage of this procedure were 7 min, 22 s (U_{max1} at 16°C); 6 min, 2 s (U_{max2} at 16°C); 6 min, 43 s (U_{max1} at 25°C); and 5 min, 16 s (U_{max2} at 25°C). At least 3 d separated the test at the acclimation temperature and the test at the elevated temperature for each fish (giving the opportunity to recover and feed at least once). Half of the fish from each population were tested initially at the elevated temperature, and half were tested initially at the acclimation temperature in order to control for any effects of learning or incomplete recovery between trials. Following the completion of all tests, a fin clip was taken from each fish to genetically identify whether it was a hybrid or one of the parental species using a DNA-based diagnostic method (see Binet and Angers 2005).

Data Analysis

All analyses were performed using the R programming language (R Development Core Team 2010). None of the lakes that we sampled contained populations of both parental species. Consequently, comparisons of swimming performance between coexisting northern redbelly and hybrid dace populations (three lakes) were analyzed separately from comparisons of swimming performance between coexisting finescale and hybrid dace populations (one lake). The three-lake comparison between coexisting northern redbelly and hybrid dace populations provided enough lake-level replication to draw general conclusions about the difference in swimming ability between these fishes. Although only one lake was available for the comparison between coexisting finescale and hybrid dace populations, the results of the analyses for this comparison are nonetheless important in identifying trends of potential general relevance.

The swimming ability of all fish was analyzed in units of body lengths per second (BL/s). In order to account for the effect of size on swimming ability, the slope of the effect of fork length on U_{max} was calculated from a linear mixed effects model with temperature, repeat swim, and lake (where applicable) as random effects. The slope was then used to adjust measurements of U_{max} to remove the effect of fish size. All subsequent analyses were performed on this size-adjusted U_{max} (BL/s).

In order to test the hypothesis that asexuals should display hybrid disadvantage, linear mixed effects models were used to analyze the (fixed) effects of species (hybrid vs. parental dace), temperature (16° vs. 25°C), and repeat swimming (U_{max1} vs. U_{max2}) on swimming performance (Table 1). In these models, because $U_{\max}$ was measured four times for each individual (U_{max1} and U_{max2} at both 16° and 25°C), individuals were treated as random effects to control for individual-level differences. In order to provide a more clear comparison of the swimming abilities of hybrid and parental dace when confronted with multiple stresses (i.e., increased temperature and exhaustion), linear models were also used to compare $U_{\max}$ under the most stressful condition (U_{max2} at 25°C) as a proportion of $U_{\max}$ under the least stressful condition (U_{max1} at 16°C). In these models, species was the only fixed effect, and lake was included as a random effect only for the comparison of northern redbelly and hybrid dace.

Results

There were differences in body size between hybrid and parental dace, and $U_{\max}$ declined significantly with increasing body size (Fig. 1). The values of $U_{\max}$ were therefore size-adjusted for subsequent analyses. The mean fork length (±SE) of northern redbelly dace from populations used in these tests of repeat swimming performance was 5.50 ± 0.37 cm, and mean fork length of finescale dace was 6.99 ± 0.60 cm. Mean fork lengths of hybrids in populations coexisting with northern redbelly dace and finescale dace were 6.53 ± 0.52 cm and 7.60 ± 0.63 cm, respectively. The linear slopes of the relationship between $U_{\max}$ (BL/s) and fork length were similar for the lake with populations of finescale and hybrid dace (slope $= -1.16$, df $= 26$, $F = 39.2$, $P < 0.0001$) and the group of lakes with populations of northern redbelly and hybrid dace (slope $= -1.15$, df $= 63$, $F = 62.2$, $P < 0.0001$). The slope of the relationship did not differ significantly between parental and hybrid populations for either lake type (redbelly-hybrid lakes: df $= 63$, $F = 1.8$, $P = 0.188$; finescale-hybrid lake: df $= 26$, $F = 0.9$, $P = 0.356$). We explored the possibility that a nonlinear model was a better fit to the data by fitting a function of the form $U_{\max} = [1/length(a)] + b$ (i.e., estimating the a and b parameters; Fig. 1). The nonlinear model was not a substantially better fit to the data in the redbelly-hybrid lakes (Δlog likelihood $= 0.67$; ΔAIC $= -1.33$) and provided no improvement in the finescale-hybrid lake (Δlog likelihood $= -0.85$; ΔAIC $= 1.71$). Therefore, for simplicity, the linear slopes were used to compute the size-adjusted $U_{\max}$ values for all subsequent analyses.

Repeat swimming and increased temperature generally reduced swimming performance. The recovery ratios (i.e., $U_{\mathrm{max2}}/U_{\mathrm{max1}}$) were calculated for repeat swimming performance tests at 16° and 25°C. At 16°C (i.e., the acclimation temperature), northern redbelly dace had a mean recovery ratio (±SE) of 0.81 ± 0.04, and the hybrids coexisting with northern redbelly dace had a mean recovery ratio of 0.81 ± 0.04. Finescale dace and their coexisting hybrids had mean recovery ratios of 0.95 ± 0.08 and 0.91 ± 0.04, respectively, at 16°C. At 25°C, northern redbelly dace and their coexisting hybrids had mean recovery ratios of 0.87 ± 0.03 and 0.84 ± 0.05, respectively, and mean recovery ratios for finescale dace and their coexisting hybrids were 0.87 ± 0.11 and 0.75 ± 0.05, respectively. To explore the potential for species bias in behavioral motivation (or willingness to perform), we analyzed contingency tables to compare the proportion of individuals of each species for which repeat swim performance was greater than initial performance (i.e., recovery ratio >1). For the redbelly-hybrid lakes, six of

Table 1: Model summary and ANOVA results for linear mixed effects models of the effects of repeat swimming and temperature on swimming performance compared between populations of hybrid and parental dace

	Comparison between *Phoxinus eos* and Hybrid Populations from Pondweed Lake, Sunday Creek, and Strubel Lake			Comparison between *Phoxinus neogaeus* and Hybrid Populations from Alford Lake		
Model:						
Fixed effects	Species × temperature × repeat			Species × temperature × repeat		
Random effects[a]	Lake/individual/temperature/repeat			Individual/temperature/repeat		
	df	*F*	*P*	df	*F*	*P*
Main effects:						
Species	66	.95	.333	28	.88	.357
Temperature	68	9.34	**.003**	28	9.88	**.004**
Repeat	68	101.19	**<.001**	28	29.70	**<.001**
Interaction effects:						
Species × temperature	68	2.71	.104	28	8.23	**.008**
Species × repeat	68	.11	.741	28	1.24	.276
Temperature × repeat	136	1.00	.319	56	6.67	**.013**
Species × temperature × repeat	136	1.06	.306	56	.39	.537

Note. The organization of parameters in the models (as either fixed or random effects) is indicated in the top rows, and the effects (either alone or in an interaction) of each parameter with a fixed effect are evaluated in the bottom rows. Significant effects are shown in bold.

[a] Slashes separate nested effects.

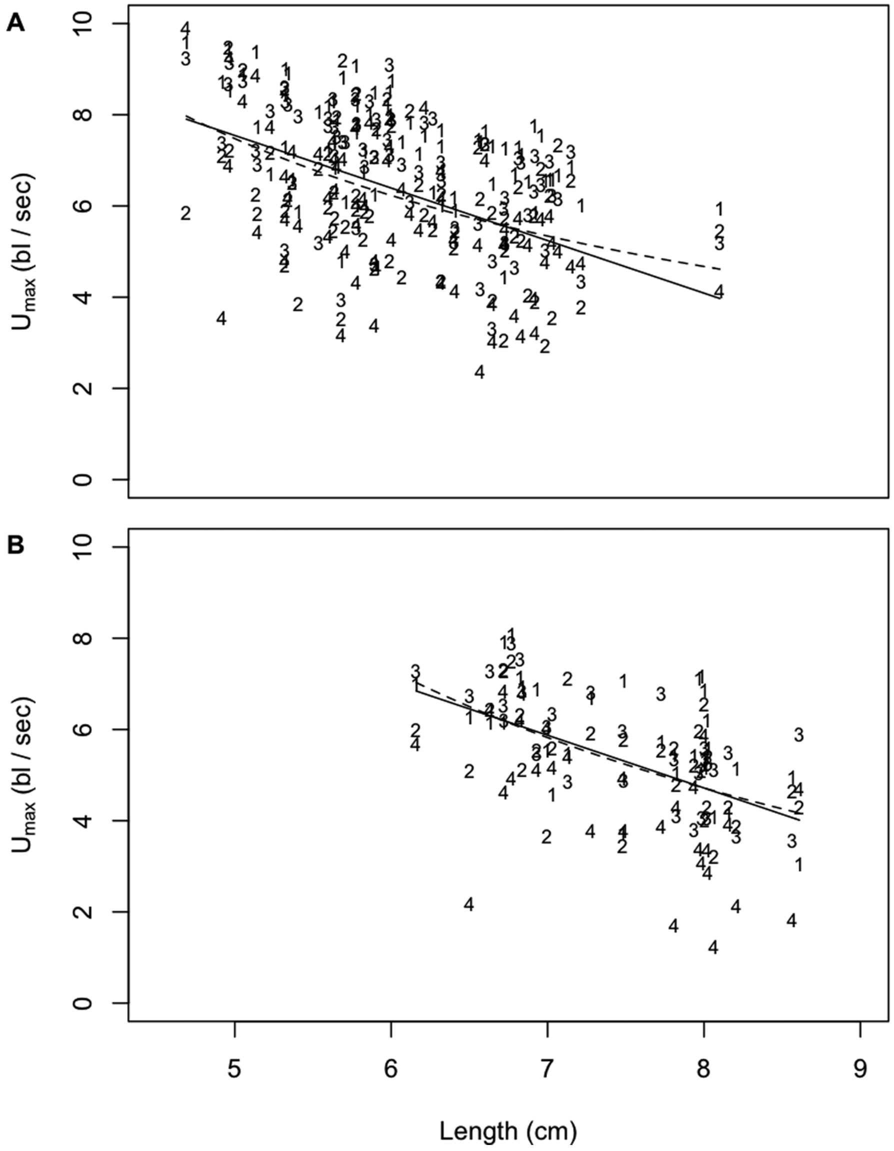

Figure 1. Relationship between size (fork length, cm) and U_{max} (body lengths/second) for northern redbelly dace and coexisting hybrids (*A*) and for finescale dace and coexisting hybrids (*B*). Number characters are used to plot the measurements, and these refer to the stages (not necessarily chronological; see main text) of the experimental procedure: *1*, U_{max1} at 16°C; *2*, U_{max2} at 16°C; *3*, U_{max1} at 25°C; *4*, U_{max2} at 25°C. Estimates of the linear and nonlinear relationships (see main text) are represented by solid and dashed lines, respectively.

the 31 parentals and six of the 39 hybrids had recovery ratios >1 at 16°C ($\chi^2 = 0.01$, $P = 0.9$), and seven of the 31 parentals and seven of the 39 hybrids had recovery ratios >1 at 25°C ($\chi^2 = 0.03$, $P = 0.9$). For the finescale-hybrid lake, two of the six parentals and five of the 24 hybrids had recovery ratios >1 at 16°C (Fisher's exact test, odds ratio = 1.9, $P = 0.6$), and one of the six parentals and four of the 24 hybrids had recovery ratios >1 at 25°C (Fisher's exact test, odds ratio = 1, $P = 1$). Therefore, there was no detectable species bias in behavioral motivation.

Linear mixed effects models revealed that there was a significant negative effect of both temperature and repeat swimming on U_{max} in the redbelly-hybrid comparison and in the finescale-hybrid comparison (Table 1; Fig. 2). A significant interaction between temperature and repeat swimming was detected only for the finescale-hybrid comparison such that repeat swimming had a more negative effect at the higher temperature; this was not the case for the redbelly-hybrid comparison (Table 1; Fig. 2). We detected no significant difference between species in the redbelly-hybrid comparison, but we found a significant interaction between species and temperature in the finescale-hybrid comparison such that temperature had a greater effect on hybrid than on finescale dace (Table 1; Fig. 2). A similar pattern was evident in the comparison of U_{max} between species under the most stressful conditions as a proportion of U_{max} under the least stressful conditions (Fig. 3). The combination

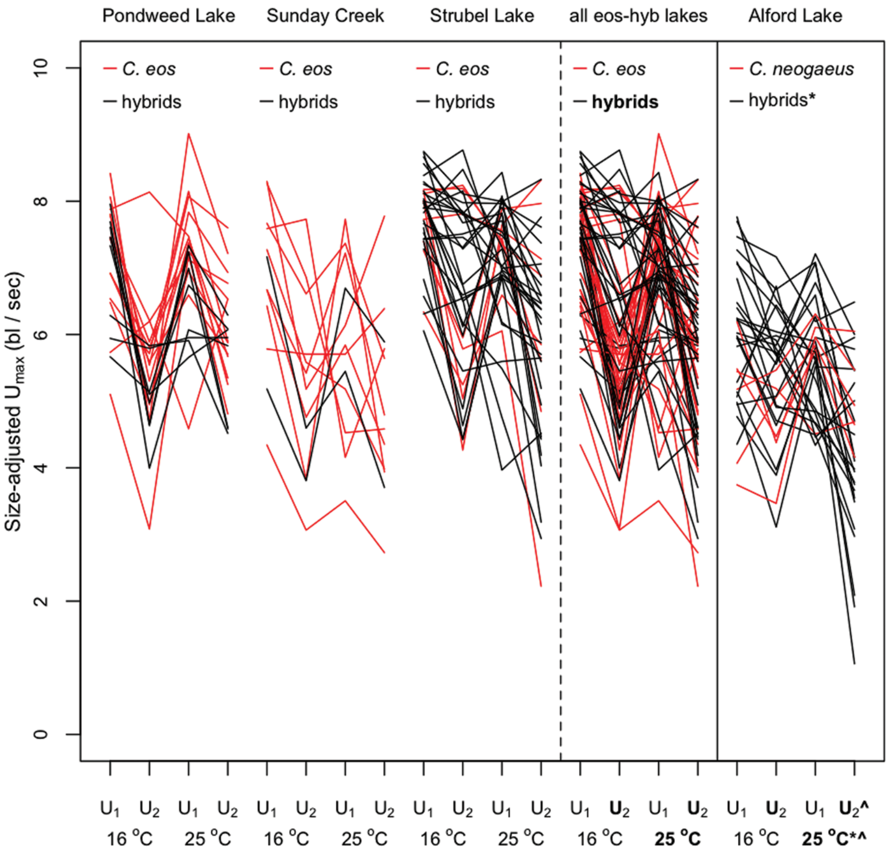

Figure 2. Effects of repeat swimming and temperature on swimming performance (U_{max}) of hybrid and parental dace from four lakes. Each line connects measurements of U_{max} at various stages of the experiment for a single individual (not necessarily chronologically; see main text). Black lines represent hybrids, and red lines represent parental species. Individuals from the three lakes represented separately to the left of the vertical dashed line are represented again together immediately to the right of the vertical dashed line. Bold labels indicate statistically significant main effects ($P < 0.05$). Shared superscript symbols (asterisk or circumflex) indicate a statistically significant interaction ($P < 0.05$).

of repeat swimming and increased temperature had the same effect for both species in the redbelly-hybrid comparison ($df = 66$, $F = 0.7$, $P = 0.404$; Fig. 3), but hybrids were more adversely affected than finescale dace in Alford Lake ($df = 28$, $F = 6.6$, $P = 0.016$; Fig. 3).

Discussion

A comparison of repeat swimming performance between asexual hybrid dace and their sexually reproducing parental species revealed that asexual hybrids performed worse relative to at least one of the sexually reproducing parental species. Asexual hybrid dace performed significantly worse than coexisting finescale dace in a repeat swim following an acute increase in temperature (Figs. 1, 2). There was also a trend, although not statistically significant, of reduced repeat swimming performance of asexual hybrids relative to coexisting northern redbelly dace (Fig. 3). Our study, while suggestive of lower repeat swimming performance of hybrids relative to parentals, strongly suggests that at a minimum, hybrid dace possess no advantages over parental species in repeat swimming performance.

Results from previous studies comparing swimming performance of hybrid and parental fishes are mixed with regard to evidence for hybrid vigor or hybrid disadvantage. Rouleau et al. (2010) compared U_{crit} at acclimation temperature (10°, 15°, or 20°C) of hybrid and pure littoral and pelagic brook trout (*Salvelinus fontinalis*) and found that hybrids performed worse at all temperatures. In a comparison of U_{crit} at acclimation temperature (21°–29°C), Rosenfield et al. (2004) showed that swimming performance of hybrids between Pecos pupfish (*Cyprinodon pecosensis*) and sheepshead minnows (*Cyprinodon variegatus*) was intermediate between the performance of the parental species.

As expected, all species of dace showed reduced U_{max} when water temperature was increased above acclimation temperature to a value likely approaching an upper temperature limit. Although an upper lethal temperature was not determined in this study, fish could not consistently swim at temperatures higher than 25°C. This overall reduction in swimming performance at higher temperature is expected when swimming is fueled aerobically—an elevation in temperature reduces oxy-

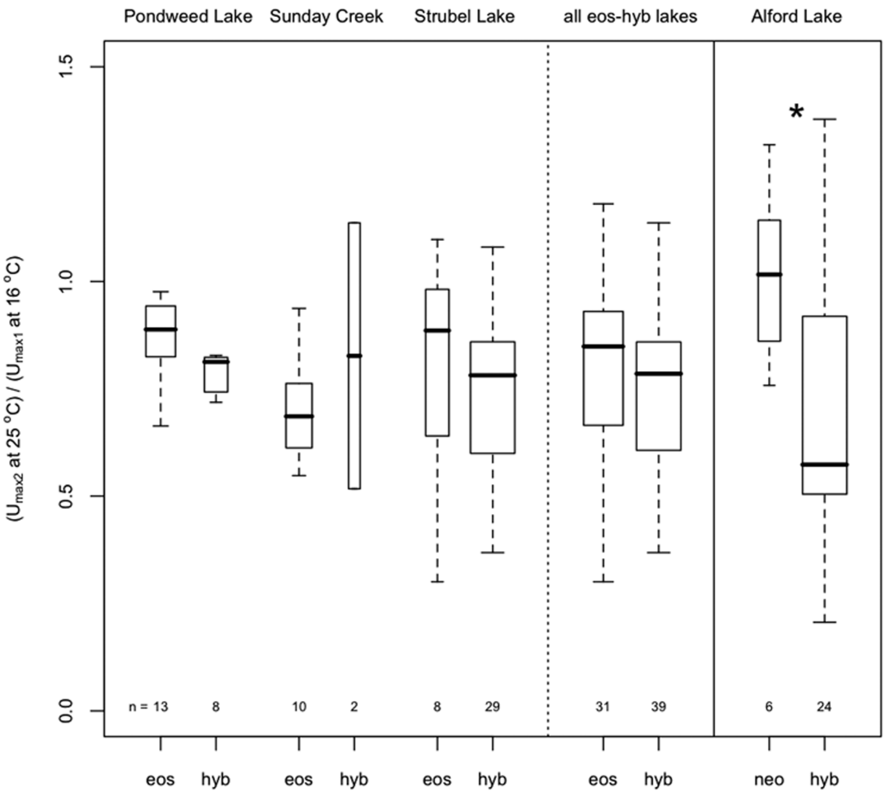

Figure 3. Swimming performance of hybrid and parental dace under the most challenging conditions (U_{max2} at 25°C) as a proportion of performance under the least challenging conditions (U_{max1} at 16°C). Horizontal bars, boxes, and whiskers give median values, second quartiles, and third quartiles, respectively, of the data for hybrid and parental dace populations. The width of boxes is proportional to the sample sizes in each population, and the sample size is given below each box. Populations from the three lakes represented separately to the left of the vertical dashed line are pooled in the lake represented immediately to the right of the vertical dashed line. A significant difference ($P < 0.05$) between populations of hybrid and parental dace (*eos* = *Phoxinus eos* population; *hyb* = hybrid population; *neo* = *Phoxinus neogaeus* population) is indicated by an asterisk.

gen-carrying capacity of the blood and oxygen solubility of the water (Randall and Brauner 1991). Anaerobically fueled swimming may also be affected when water approaches lethal temperatures, but data on the effect of temperature on the anaerobic component of swimming performance are lacking.

Our study provides some evidence that repeat U_{max} is reduced by acute changes in temperature, but the mechanism of the effect (i.e., whether it involves aerobic or anaerobic processes) is not known. Finescale dace and the hybrid dace from Alford Lake performed worse in a repeat swim at elevated temperature than at acclimation temperature. This was not, however, the case for dace populations from the other lakes. To our knowledge, there are no other studies of the effects of acute temperature changes on repeat swimming performance in fish. Some studies have shown that recovery from exhaustion occurs faster at higher temperatures in salmonids (Kieffer et al. 1994; Wilkie et al. 1997; Kieffer 2000), and one study of repeat swimming performance in rainbow trout (*Oncorhynchus mykiss*) showed that recovery ratios decreased at warmer temperatures (Jain and Farrell 2003), although fish in all these studies were only tested at temperatures to which they were acclimated. Jain and Farrell (2003) suggested that the reduced recovery ratios at warmer temperatures occurred because fish swim faster and harder on their first swim and hence were unable to recover as quickly compared with repeat swimming at colder temperature, where the fish were less active and did not swim as hard on their first swim. This hypothesis, however, cannot apply to the decrease in recovery ratio at higher temperature in the case of finescale and hybrid dace from Alford Lake because U_{max1} was lower at the higher temperature than at the acclimation temperature and thus likely represents a direct effect of temperature on repeat U_{max}. Given that this test likely has a large anaerobic component, this may indicate that a greater degree of glycogen depletion was observed following U_{max1} at high temperature (Farrell 2008); however, further studies are required to address this. If hybrids have reduced overall physiological performance relative to the parental species, this might explain the effect of temperature on repeat performance of hybrids.

Swimming ability is just one aspect of the ecological and physiological performance of fish that contributes to fitness. Additional measurements of other components are required to more fully characterize the relative fitness of asexual and sexual dace. One such measurement, conducted by Mee and Rowe (2006), showed that asexual dace carried heavier parasite loads than sexual dace. Further, Das and Nelson (1990) measured the fecundity of asexual and sexual dace and found no differences in this proxy measure of fitness. Taken together, these studies suggest a lack of hybrid vigor and possibly the existence of hybrid disadvantage among asexual dace. The lack of hybrid vigor, and especially the existence of hybrid disadvantage, would facilitate the coexistence of asexual and sexual dace by offsetting the advantages inherent in asexual reproduction (Moore 1976; Hellriegel and Reyer 2000; Mee and Otto 2010).

It is likely that our test of a fish's ability to repeat a high-intensity swim following an acute temperature change is a good indicator of overall physiological performance (Jain et al. 1998). This could be tested directly by using methods similar to ours to compare repeat swimming performance among fish carrying various parasite loads or exposed to different levels of pollutants. Despite the lack of such confirmation, our data showing that hybrid dace performed worse than at least one of the parental species suggest that the hybrids do have reduced physiological performance. The use of physiological performance as a proxy for fitness might be conservative in terms of ecological relevance, especially if the previous evidence of increased parasite loads on hybrid fish (Mee and Rowe 2006) are considered. These two measures of ecological and physiological performance, both reduced in hybrid dace, suggest that a multitude of factors, perhaps acting synergistically, could result in much greater performance of parental species relative to hybrid dace under natural conditions. A combination of multiple factors and multifarious selection has been shown to decrease hybrid fitness in other systems. For example, hybrid stickleback (*Gasterosteus aculeatus*) have been shown to grow more slowly (Hatfield and Schluter 1999) and have lower juvenile survival (Vamosi et al. 2000) than pure benthic or limnetic species. Similarly, hybrid whitefish (*Coregonus clupeaformis*) have increased embryonic mortality and more asynchronous emergence relative to pure dwarf or normal ecotypes (Rogers and Bernatchez 2006). Hybrids between *Drosophila gaucha* and *Drosophila pavani* are not only sterile but have reduced larval viability and inferior foraging and locomotory behavior (Brncic and Budnik 1974; Godoy-Herrera et al. 2005).

The fitness of asexuals and sexuals has been compared in other asexual vertebrate systems, and the evidence is mixed with regard to the existence of hybrid disadvantage or hybrid vigor. Hybrid disadvantage has been detected in a number of asexual systems, including geckos (Moritz et al. 1991), *Cnemidophorus* lizards (Cullum 1997), European wood frogs (Vorburger 2001), and poeciliid fish (Wetherington et al. 1987; Lively et al. 1990; Weeks 1995), while hybrid vigor has been detected in asexual geckos (Kearney et al. 2005) and asexual European wood frogs (Hotz et al. 1999). Additional studies comparing fitness-related traits between asexual and sexual species in dace and other hybrid asexual systems will continue to provide information on the balance of advantages and disadvantages of sexual and asexual reproduction. Such studies are needed to resolve further the reasons for the rarity of asexual reproduction among vertebrates.

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