Alternative male reproductive tactics drive asymmetrical hybridization between sunfishes (*Lepomis* spp.)

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The potential role of alternative reproductive tactics in circumventing premating isolating mechanisms and driving hybridization between species has long been recognized, but to date there is little empirical support from natural systems. Hybridization occurs between bluegill (*Lepomis macrochirus*) and pumpkinseed sunfish (*Lepomis gibbosus*) and it is known to be asymmetrical (male bluegill × female pumpkinseed). Here, we test whether this pattern is driven by a recognition failure by pumpkinseed females or by an alternative cuckold reproductive tactic in bluegill males. Using genetic parentage data, we found that bluegill cuckolders fathered 24.9% of the larvae in bluegill nests, but no evidence that pumpkinseed females spawned in bluegill nests. Pumpkinseed cuckolders fathered 8.7% of the larvae in pumpkinseed nests, whereas bluegill cuckolders fathered 13.6% of the larvae in those nests. Bluegill cuckolders thus frequently spawn in pumpkinseed nests and are responsible for the asymmetrical hybridization between the species. We discuss the evolutionary consequences of interactions between bluegill and pumpkinseed and the role of alternative reproductive tactics in adaptation and introgression.

1. Introduction

Alternative reproductive tactics are an important component of mating systems in all major taxonomic groups [1]. For example, in sunfish (*Lepomis* spp.) most males mature late in life and use a parental tactic: they construct nests where they spawn with females, then provide sole parental care to the developing eggs. Other males mature early in life and use a cuckolder tactic: they fertilize eggs by releasing sperm in the nest of a parental male while a female is spawning. Specifically, small cuckolders act as ‘sneakers’ that dart into the nest at the moment of spawning, whereas large cuckolders act as ‘satellites’ that deceive parental males by mimicking female characteristics [2]. This variation has led sunfish to become a model system for the study of alternative reproductive tactics [2,3].

The presence of alternative reproductive tactics has consequences for the evolution of mating systems. In sunfish, for example, cuckolders diminish the opportunity for mate choice. Females can choose a parental male as a mate, but that choice is often negated by a cuckolder intrusion during spawning [4]. Sperm competition and agonistic interactions with parental males mean that cuckolders must rapidly enter a nest [5], which may limit the opportunity for mate choice by cuckolders. In the most extreme cases, it is conceivable that cuckolders do not effectively discriminate among sunfish species. Consequently, it has been suggested that cuckolder males may facilitate interspecies hybridization in sunfish and in other taxonomic groups [6,7], but to date there is little empirical evidence that cuckolders drive hybridization patterns in natural systems.

Bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) sunfish frequently hybridize in Lake Opinicon [7]. Based on allozyme loci and mitochondrial
DNA, all hybrids appear to be first generation (F1) and have a pumpkinseed mother [7]. This unidirectional hybridization could be a result of mate recognition errors by either pumpkinseed females or bluegill cuckolders. Here, we examine the relative contributions of pumpkinseed females and bluegill cuckolders to hybridization patterns by measuring the abundance of hybrid offspring in the nests of bluegill and pumpkinseed males. If hybridization is driven primarily by pumpkinseed females, then hybrids should be abundant in bluegill nests but rare in pumpkinseed nests. If hybridization is driven primarily by bluegill cuckolders, then hybrids should be rare in bluegill nests but abundant in pumpkinseed nests.

2. Material and methods

(a) Sample collection
All sunfish were collected in Lake Opinicon, Ontario, Canada (44°34’ N, 76°19’ W). Parental males were captured using dip nets, and a small tissue sample from the male and approximately 25 larvae from his nest were preserved in 95% ethanol. Bluegill nests were sampled in 2005 (n = 37, described in [8]), and additional parental males were sampled in 2010 (n = 22) for the interspecies comparisons of allele frequencies. Pumpkinseed nests were sampled in 2003 (n = 12, described in [4]) and 2010 (n = 37).

(b) Genetic analysis
Each individual was genotyped at three microsatellite loci: RB7, LMAR10 and either RB20 or LMAR14 (for details of these loci see [9,10]). RB20 was used for the parentage analysis of the 2005 bluegill samples, whereas LMAR14 was used for the interspecies allele frequency comparisons and the parentage analysis of the 2003 and 2010 pumpkinseed samples.

We identified microsatellite loci that were informative for species identification by comparing the distribution of allele sizes in parental males from each species. We found that LMAR10 and LMAR14 were each partially informative for species identification, and in combination would correctly identify 100% of pure bluegill, 100% of pure pumpkinseed and 97.6% of hybrids (detailed calculations are available in the electronic supplementary material). These loci were then used to assign each larva as a bluegill, pumpkinseed or hybrid. Additionally, for a small number of larvae with ambiguous ancestry (n = 9), we used a mitochondrial restriction assay (described in the electronic supplementary material) to determine whether the female parent was a bluegill or pumpkinseed.

For each nest, we calculated the proportion of paternity that could be attributed to the parental male and to cuckolders of each species. Hybrid larvae have low probability of being genetically compatible with a parental male by chance, so paternity for these larvae was assigned based on simple exclusion. For the remaining larvae, the share of paternity for the parental male and for cuckolders of his species was determined using the two-sex paternity model [11]. In pumpkinseed nests, the paternity of pumpkinseed parental males, pumpkinseed cuckolders and bluegill cuckolders did not differ significantly between 2003 and 2010 (t-tests, all p > 0.05), and data from these years were thus combined in our analyses.

3. Results
We genotyped a total of 865 larvae to measure paternity in the nests of 37 bluegill males (mean = 23.4 larvae per nest, range = 10–48). We found that the average parental male bluegill sired 75.1% of the larvae in his nest (figure 1a). Paternity for the remaining 24.9% of the larvae was always assigned to bluegill cuckolders. Pumpkinseed ancestry was not inferred for any of the larvae in bluegill nests.
We genotyped 1169 larvae to measure paternity in the nests of 49 pumpkinseed males (mean = 23.9 larvae per nest, range = 12–26). We found that the average parental male pumpkinseed sired 77.7% of the larvae in his nest (figure 1b). Paternity for 8.7% of the larvae in pumpkinseed nests was attributed to cuckoldry by other pumpkinseed males, while paternity for the remaining 13.6% of the larvae was attributed to cuckoldry by bluegill males. Overall, there were 160 larvae in pumpkinseed nests for which we inferred bluegill ancestry: 151 larvae (94.4%) did not match the nest-tending parental male pumpkinseed and had microsatellite genotypes that were consistent with an F1 hybrid produced by a bluegill cuckolder and pumpkinseed female. One larva (0.6%) had two different bluegill alleles at both informative microsatellite loci and a bluegill mitochondrial haplotype, consistent with a mating between a bluegill cuckolder and bluegill female. Three larvae (1.9%) had two different bluegill alleles at one microsatellite locus and a pumpkinseed mitochondrial haplotype, consistent with a backcross between a bluegill cuckolder and a hybrid female. The remaining five larvae (3.1%) were a genetic match for the nest-tending parental male pumpkinseed, but had both bluegill and pumpkinseed microsatellite alleles and a pumpkinseed mitochondrial haplotype, consistent with either a backcross between the nest-tending parental male pumpkinseed and a hybrid female, or with a chance genetic match between the pumpkinseed parental male and a hybrid produced by a bluegill cuckolder and pumpkinseed female. 


10. Schable NA, Fischer RU, Glenn TC. 2002 Tetranucleotide microsatellite DNA loci from the...


