Does a predatory insect contribute to the divergence between cave- and surface-adapted fish populations?

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Immigrant inviability, where individuals from foreign, ecologically divergent habitats are less likely to survive, can restrict gene flow among diverging populations and result in speciation. I investigated whether a predatory aquatic insect (*Belostoma* sp.) selects against migrants between cave and surface populations of a fish (*Poecilia mexicana*). Cavefish were more susceptible to attacks in the light, whereas surface fish were more susceptible in darkness. Environmentally dependent susceptibility to attacks may thus contribute to genetic and phenotypic differentiation between the populations. This study highlights how predation—in this case in conjunction with differences in other environmental factors—can be an important driver in speciation.

Keywords: ecological speciation; immigrant inviability; local adaptation; predator–prey interaction; reproductive barriers

1. INTRODUCTION

Exposure to different environments often leads to adaptive trait divergence (Schluter 2001). Divergent selection may result in reduced interpopulation gene flow, and reproductive isolation may evolve as a by-product of local adaptation (Schluter 2001; Rundle & Nosil 2005). This phenomenon has been documented in several natural systems (e.g. Grant 1993; Nosil & Crespi 2006a; Langerhans *et al.* 2007) as well as in laboratory evolution experiments (Rundle *et al.* 2005; Dettman *et al.* 2007).

Reproductive isolation is central to the speciation process and can be achieved via several routes (Coyne & Orr 2004). Generally, reproductive isolating mechanisms may be prezygotic, where potential mates either do not meet (temporal isolation, Lamont *et al.* 2003; habitat isolation, Via 1999), are not attracted to each other (Vines & Schluter 2006), or are incompatible during mating or fertilization (Jordal *et al.* 2006; Ludlow & Magurran 2006). Alternatively, they can be postzygotic, where hybrids are inviable (Wu & Ting 2004) or selected against by natural selection (Hatfield & Schluter 1999) or sexual selection (Naisbit

et al. 2001). One of the simplest mechanisms of pre-mating reproductive isolation is immigrant inviability (Hendry 2004; Nosil *et al.* 2005), where immigrants from ecologically divergent habitats experience disproportionately higher mortality and have a lower probability of successfully reproducing.

Predation may play a pivotal role during ecological speciation. Predators have been shown to cause adaptive trait divergence directly among lineages, and hence drive diversification and speciation (Fryer 1959; Jiggins *et al.* 2001; Mikolajewski *et al.* 2006; Nosil & Crespi 2006b; Langerhans *et al.* 2007). In this study, I investigated whether selection against immigrants caused by a predatory aquatic insect could contribute to the differentiation between two parapatric populations of surface- and cave-dwelling fish.

In the Cueva del Azufre system of southern Mexico, the livebearing fish *Poecilia mexicana* (Atlantic molly, Poeciliidae), which is widespread in surface streams in Central America, has colonized subterranean habitats. Cave and surface populations have diverged phenotypically, with cavefish having reduced eyes and pigmentation and more elaborate non-visual senses (Parzefall 2001). Despite the lack of major physical barriers to dispersal and the spatial proximity of cave and surface populations, they are differentiated genetically and phenotypically (Tobler *et al.* 2008a). To date, it is unclear what mechanisms maintain the high level of differentiation between cave and surface populations.

Giant water-bugs of the genus *Belostoma* (Hemiptera, Belostomatidae) are efficient predators and occur in both the Cueva del Azufre and adjacent surface habitats (Tobler *et al.* 2007). *Belostoma* have been shown to preferentially attack certain sizes and sexes of prey, and thus may exert strong selective pressures on prey species (Tobler *et al.* 2007, 2008b). Similarly, immigrants to cave and surface habitats could be attacked selectively, as cave and surface fish have diverged in sensory structures (Parzefall 2001). Consequently, predation by *Belostoma* could promote divergence between populations of cave- and surface-adapted *P. mexicana*. Here, I simultaneously exposed fish from surface and cave populations to *Belostoma* to test whether attack rates differ between surface and cavefish depending on habitat.

2. MATERIAL AND METHODS

Experiments were conducted in August 2008 and February 2009 in the Cueva del Azufre system (17.442° N, 92.775° W) near Tapijulapa (Tabasco, Mexico). Cavefish were collected in the front chambers of the Cueva del Azufre and surface fish at the cave resurgence (figure 1a). *Belostoma* were collected in the front chambers of the cave. Immediately after capture, a cavefish, a surface fish and a *Belostoma* were placed into a 2 l plastic bottle that had been perforated with approximately 30 small holes to allow for water and air exchange during the experiment. The bottles adequately mimicked the natural conditions, as the fish and their predator occur in tight and shallow spaces in this system (figure 1b,c). Because male (Tobler *et al.* 2008b) and large-sized fish (Tobler *et al.* 2007) are more susceptible to *Belostoma* attacks, fish in each bottle were matched for sex and size. Bottles were then placed in a shallow area either within the cave or at a shaded spot at the cave resurgence, and fixed in place with rocks. Bottles were partially (approx. 80%) submerged to allow the water-bugs to breathe and fish to perform aquatic surface respiration (Plath *et al.* 2007).

After 24 h, *Belostoma* attacks were quantified by checking all fish for puncture wounds and assessing mortalities. The standard length of fish and the total length of *Belostoma* were measured to the closest millimetre using callipers (see electronic supplementary material). All surviving fish and water-bugs were released at their original collection sites after the experiment was terminated. The experiment

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Figure 1. (a) Simplified map of the study location (light grey areas, water; dark grey areas, bedrock; small arrows, direction of flow; X, cave exit; dashed ellipses, collecting area of study organisms; asterisks, location of bottles). (b) Fish aggregate in high densities in shallow, relatively tight spaces. (c) *Belostoma* lurk close to the water surface for bypassing prey.

consisted of 32 replicates in each habitat. I tested for differential susceptibility to *Belostoma* attacks between surface and cavefish by applying a Fisher's exact test. Data were analysed using SPSS 16 (SPSS Inc., Chicago, IL, USA).

In addition to the standard predation experiment, I performed two additional experiments. In one, fish were added to the bottle and given 24 h to acclimatize to experimental conditions (15 replicates in each habitat). Then a *Belostoma* was added, and the experiment was continued as described earlier. Also, a control experiment was conducted as described earlier, but without adding a predator to a bottle (15 replicates in each habitat) to estimate background mortalities (e.g. owing to handling stress).

3. RESULTS

Belostoma attacks on fish were recorded in 48 of the 64 bottles (23 from the surface treatment, 25 from the cave treatment). In seven trials, both surface and cave individuals exhibited puncture wounds. Predator attacks on cave and surface fish in the two habitats significantly differed from random expectations ($n = 55$, $p < 0.001$). Cavefish were attacked more than surface fish at the surface, whereas surface fish were attacked more in the cave (figure 2a). This pattern remained the same when fish were given 24 h for acclimatization prior to the predator exposure. *Belostoma* attacks were found in 20 of the 30 trials (10 in each treatment; whereas both fish exhibited puncture wounds in one surface trial). Again, cavefish were attacked more at the surface and vice versa ($n = 21$, $p = 0.03$; figure 2b); however, owing to the limited sample size, this result has only low statistical power.

Attacks by *Belostoma* probably result in high mortality of attacked fish as 35 per cent of individuals with puncture wounds died from their injuries within the time of the experiment. In contrast, no background

mortality was recorded in the control experiment without a predator.

4. DISCUSSION

Susceptibility to *Belostoma* attacks in *P. mexicana* of the Cueva del Azufre system is environmentally dependent. Cavefish were more susceptible in surface habitats, whereas surface fish were more susceptible to predator attacks within the cave. This difference is probably attributable to differences in sensory structures between cave and surface fish. Cavefish exhibit reductions in eye size (Tobler *et al.* 2008a), opsin gene expression (S. W. Coleman, M. Tobler & G. G. Rosenthal 2008, unpublished data) and optomotoric responses (Parzefall *et al.* 1997), but have hypertrophied gustatory and mechanosensory organs (Parzefall 2001), which allow for communication and orientation in darkness (Plath *et al.* 2004). Thus, they should be able to better detect predators in darkness where non-visual cues are important in predator detection. On the other hand, surface fish should have an advantage in the light. Early detection of predators is crucial in successfully evading capture in most predator-prey systems (Krause & Godin 1996; Lind 2004). It remains to be studied to what extent cave and surface fish's ability to avoid *Belostoma* attacks under different environmental conditions is phenotypically plastic, as experience can alter anti-predator responses (Kelley & Magurran 2003). However, the use of visual and non-visual senses in other contexts (e.g. during mate choice) has been shown to have a strong genetic basis in cave as well as surface populations (Plath *et al.* 2004).

Figure 2. (a) Cavefish (black bars) were more susceptible to *Belostoma* attacks in the light, whereas surface fish (grey bars) were more susceptible in the dark. (b) This pattern remains when fish are given a 24 acclimatization period before the predator is added.

Predation has previously been shown to cause adaptive trait divergence and promote ecological speciation (Jiggins *et al.* 2001; Vamosi 2005; Nosil & Crespi 2006b; Langerhans *et al.* 2007). In conjunction with the different light environments (Tobler *et al.* 2008a), predation by *Belostoma* may be a driving factor in the divergence of cave and surface populations of *P. mexicana*. Overall, these results are consistent with the idea that divergent selection by predation can cause immigrant inviability and contribute to reproductive isolation; however, future studies will need to address whether attack rates and mortalities in experiments can be extrapolated to natural conditions, and to what extent selection by *Belostoma* counteracts migration between the populations (also see the electronic supplementary material). Divergence between populations is unlikely to be driven by predation alone, but habitat differences in resource availability, competitive regime or social environment may exert divergent selection as well. Consequently, the next challenge will be to identify independent axes of trait divergence and selection and to determine whether, and to what degree, different mechanisms of reproductive isolation, which could act in concert, contribute to the speciation process (Nosil *et al.* 2009).

For the collection of these data the author adhered to the Guidelines for the Use of Animals in Research. The experiments reported here are in agreement with the respective laws in Mexico (DGOPA.06192.240608-1562).

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