



Figure 2

(A) Mean association times (\pm SE) of *Xiphophorus variatus* females in control experiments to determine responsiveness to the male animation and ability to choose between 2 male stimuli. A random sample of females was used for both tests. In both tests, females exhibited significant differences in association times between stimuli, denoted by asterisks ($P < 0.05$). (B) There was no evidence of either positive or negative assortative preferences of females for tailspot patterns. Regardless of tailspot type, females spent equal time with both male stimuli. The images below the x axis represent the female tailspot types that were tested in each associated experiment.

demonstrate that mating outcomes are consistent with mating preferences. The results of our study contrast with findings in *X. maculatus* that show disassortative mating patterns, which suggests that, in *X. variatus*, natural selection may be the primary driver of the maintenance of the tailspot polymorphism. There was no evidence that tailspot pattern size (pigment area) is a limiting factor in eliciting female response.

Females responded positively in both control experiments, demonstrating their propensity to respond to the fish animation and to choose between pairs of male stimuli. In a previous study, female *X. variatus* preferred males of larger lateral projection area (i.e., larger dorsal fin for a fixed body size; MacLaren et al. 2011). Here, females preferred a smaller dorsal fin when body size was held constant. A number of factors could account for preference for smaller dorsal fins including recent exposure (cf., Verzijden and Rosenthal 2011) because females had been exposed to the male with the smaller dorsal fin during prior trials. Within the context of this study, however, the critical finding is that females did show a significant preference for one male over the other, demonstrating ability to attend to visual signals and choose between stimuli.

Previous investigation of mating patterns in *X. variatus* involved collecting gravid females from the wild and determining the tailspot phenotype of their offspring. This study suggested no deviation from random mating and, specifically, no evidence of disassortative mating (Borowsky and Khouri 1976). Our findings contrasted with a similar study first conducted on *X. maculatus*, which demonstrated disassortative patterns (Borowsky and Kallman 1976). Females of the wild-type, cut crescent, and crescent tailspot

types exhibited random preferences in mate choice trials, spending equal time with a male of their own tailspot type as they did with a male of a dissimilar type. It seems unlikely that nonrandom mating preferences contribute to the maintenance of the tailspot polymorphism in a meaningful way for *X. variatus*. However, one possibility is that mating and mate preferences may be frequency dependent. There is ample evidence, including examples in poeciliids, that early experience and recent exposure can alter female preference (Verzijden and Rosenthal 2011; Verzijden et al. 2012). For example, exposure to heterospecifics can either strengthen or weaken female preferences for conspecifics or even generate preference in a new direction (e.g., toward heterospecifics; Verzijden et al. 2012). The frequency of phenotypes in a population can also affect female choice, and negative frequency-dependent selection could be a mechanism favoring balanced polymorphism at the tailspot locus. Examples of negative frequency-dependent selection have been reported in 2 closely related species (Hughes et al. 1999; Royle et al. 2008). Such patterns of mating and mate preferences could be difficult to detect in *X. variatus*, because allele and pattern frequencies can change from one generation to the next owing to selection from environmental variables like temperature (Culumber et al. forthcoming).

The difference in mating patterns in natural populations between *X. variatus* and *X. maculatus* observed in the 1976 studies is puzzling. However, low sample size could make it difficult to detect such patterns. As previously pointed out, large sample sizes are likely necessary to detect nonrandom mating in *X. variatus* (Borowsky 1990). The sample of females for mating pattern inference in Borowsky

