A Behavioral Syndrome Linking Boldness and Flexibility Facilitates Invasion Success in Sticklebacks

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ABSTRACT: For a species to expand its range, it needs to be good at dispersing and also capable of exploiting resources and adapting to different environments. Therefore, behavioral and cognitive traits could play key roles in facilitating invasion success. Marine threespined sticklebacks (Gasterosteus aculeatus) have repeatedly colonized freshwater environments and rapidly adapted to them. Here, by comparing the behavior of hundreds of lab-reared sticklebacks from six different populations, we show that marine sticklebacks are bold, while sticklebacks that have become established in freshwater lakes are flexible. Moreover, boldness and flexibility are negatively correlated with one another at the individual, family, and population levels. These results support the hypothesis that boldness is favored in invaders during the initial dispersal stage, while flexibility is favored in recent immigrants during the establishment stage, and they suggest that the link between boldness and flexibility facilitates success during both the dispersal stage and the establishment stage. This study adds to the growing body of work showing the importance of behavioral correlations in facilitating colonization success in sticklebacks and other organisms.

Keywords: plasticity, persistence, novel object, intraspecific variation, convergent, rapid adaptation.

Introduction

Understanding the factors that allow a species to expand its range and adapt to changing habitats is increasingly important in the face of anthropogenic change. Natural biological invasions can reveal how and why certain organisms can excel in response to novel selection pressures (Whitney and Gabler 2008). In addition to the importance of propagule pressure (Lockwood et al. 2005), stochasticity, and the opening of new niches at the edge of species boundaries, there is growing evidence that particular traits (e.g., r-selected life histories [Capellini et al. 2015], habitat breadth [Blackburn et al. 2009], large brains/cognitive abilities [Sol et al. 2005]) might promote biological invasions. Behavioral and cognitive traits are likely to play an important role in allowing animals to move into and become established in new environments (Sih et al. 2010; Wright et al. 2010; Hostet et al. 2011; Chapple et al. 2012). For example, behavioral plasticity allows animals to rapidly adjust their phenotype in response to changes in environmental cues (Wolf et al. 2008; Coppens et al. 2010; Sih et al. 2012). Cognitive processes—how animals perceive, process, and retain information about their environment and then use that information to make decisions (Shettleworth 2010)—may play an especially important role during biological invasions because they influence the ability of animals to enter new habitats, exploit new niches, become established, and spread (Wright et al. 2010; Griffin et al. 2016; Szabo et al. 2020). For example, recent immigrants have to be willing to approach and interact with novel stimuli to gain information about their new environment (i.e., neophilia; Mettke-Hofmann et al. 2009). Additionally, previously successful behavioral patterns may no longer be successful in new environments, so immigrants need to be able to stop persisting on ineffective responses and be flexible enough to attempt new approaches (Griffin et al. 2016).

Successfully colonizing a new environment can be broken down into discrete stages (e.g., dispersal, colonization, establishment, and spread; Chapple et al. 2012), and invasion success likely relies on different behavioral and cognitive traits in each stage (Sih et al. 2012). For example, dispersers need to be bold, while immigrants can succeed in a new environment when they are willing to investigate novel stimuli and when they are able to quickly change
moved and ineffective behaviors (i.e., if they are flexible and
reactive; Koolhaas et al. 1999; Sih et al. 2012). Numerous
studies have documented differences in behavioral and
physiological traits between invading and established popu-
lations (e.g., Martin and Fitzgerald 2005; Pintor et al.
2008; Damas-Moreira et al. 2019; Cohen et al. 2020),
and a handful of studies have shown that differences are
preserved in a common garden (e.g., Sargent and Lodge
2014; Gruber et al. 2017; Liparoto et al. 2020). If there is
an underlying genetic basis to the behavioral and cognitive
traits that facilitate range expansions or biological inva-
sions, then we might expect those traits to evolve over
the course of an invasion. According to this hypothesis, be-
havioral and cognitive traits important for invasion should
vary in a systematic way between dispersing populations
compared with newly derived and well-established popu-
lations when they are reared in a common garden. More-
over, if invasion success requires different behavioral and
cognitive traits in the different invasion stages, then mecha-
nisms that package these traits together could be key to
the success of invasive species (Sih et al. 2012). For ex-
ample, traits important for the dispersal and establishment
stages are coupled together in western bluebirds, and this
facilitates the expansion of their range (Duckworth and
Badyaev 2007).

Three-spined sticklebacks (Gasterosteus aculeatus) are a
model system for studying trait evolution during biologi-
cal invasions. Throughout their evolutionary history, ma-
rine sticklebacks have repeatedly colonized freshwater
environments, rapidly adapted to them, and diversified
(Stuart et al. 2020). Sticklebacks can also spread and have
dramatic impacts on freshwater communities (Eklöf et al.
2015; Gegele et al. 2020). Work on this system has pri-
marily focused on a suite of morphological and physiolog-
cal traits that repeatedly evolve when marine sticklebacks
invade freshwater habitats (Bell et al. 2004; Colosimo et al.
2005), with evidence that haplotypes containing a set of
coadapted alleles are maintained at low frequency in the
ocean and are repeatedly tapped during adaptation to
freshwater (Bassham et al. 2018). However, little is known
about the behavioral and cognitive mechanisms that may
facilitate the invasion of sticklebacks into new habitats and
that may evolve once a population becomes established
(Foster et al. 2015).

We took advantage of the repeated invasion of fresh-
water habitats by sticklebacks by comparing marine
sticklebacks with sticklebacks from recently derived and
well-established freshwater populations in Alaska, with
replicate populations of each type. For each population,
we reared clutches of field-fertilized eggs in a controlled
laboratory environment and scored individuals for three
informative and repeatable behavioral traits: boldness, be-
havioral flexibility, and neophilia (Bensky and Bell 2020).
We hypothesized that boldness is favored in dispersers
(i.e., marine populations) and that neophilia and behav-
ioral flexibility are favored in established populations.
The replicate populations allow us to assess the generality
of the patterns and comparing newly established popu-
lations with well-established populations provides insight
into whether different traits are favored during initial es-
ablishment versus population persistence (Merwin 2019).
Rearing animals under common-garden conditions in the
lab gives us insight into whether behavioral variation within
and among populations might reflect evolved, genetically
based differences, with the caveat that parental effects could
also contribute.

Material and Methods

Adult sticklebacks were collected via minnow traps from
six populations ranging from the Matanuska-Susitna Val-
ley to the Kenai Peninsula of Alaska (fig. S1; table S1) during
June 2017. Two populations (Rabbit Slough [M] and Resur-
rection Bay [M]) occur in the ancestral marine environment
(M indicates marine), while the remaining four populations
occur in freshwater. Two of the freshwater populations (Big
Beaver [E] and Cornelius [E]; hereafter referred to as “well
established”; E indicates established) are derived from nat-
ural colonization events that presumably occurred hundreds
to thousands of years ago, after the last glacial maximum.
The other two freshwater populations are recently derived
(N indicates new) via natural recolonization (Loberg [N]:
28–34 years before collection; Bell et al. 2004) or experi-
mental seeding (Cheney [N]: 8 years before collection; Bell
2016) following deliberate rotenone poisoning, which ex-
terminated all of the fish. The fish that were introduced in-
to Cheney Lake had been caught by unbaited minnow traps
in Rabbit Slough (M; Bell 2016). It is likely that genetic di-
versity among these populations follows the pattern typical
for sticklebacks (Bell 1988), where the marine populations
are weakly genetically differentiated from each other ($F_{ST} =$
0.0076), while the freshwater populations are more strongly
genetically differentiated ($F_{ST}$ estimates between these and
nearby lakes in Alaska range from −0.05 to 0.15; Hohenlohe
et al. 2010; Roberts Kingman et al. 2021). Salmonid predators
are present in all of the populations. The two well-established
freshwater lakes are larger in terms of volume compared with
the lakes with the two new populations, but time since es-
ablishment is not confounded with average or maximum
lake depth, which is an important driver of ecological diver-
genence in sticklebacks (Willacker et al. 2010; Voje et al. 2013;
Østbye et al. 2016; table S1).

Full-sib clutches within each population were generated
via artificial fertilization and incubation. To collect sperm,
males from each population were euthanized using an overdose of buffered tricaine methanesulfonate (MS-222), and their testes were immediately dissected and macerated. Eggs were then gently extruded from gravid females from the same population into a petri dish, and the macerated testes were pipetted over the eggs to fertilize them. Distilled water with 6% Instant Ocean was used to repeatedly rinse the newly fertilized clutches before they were stored in that solution in the petri dish. The clutches were inspected daily for proper development; dead embryos and unfertilized eggs were removed, and the water was changed. Two or three days after fertilization, the eggs were transferred to 50-mL canonical tubes and shipped overnight in coolers filled with ice packs to the University of Illinois Urbana-Champaign, where they were raised in common-garden conditions in the lab. Artificial incubation controls for environmental paternal effects due to receiving paternal care. Full-sib clutches (hereafter, “families”) were reared in separate tanks (9.5 L; 32 cm × 21 cm × 19 cm), where the embryos were incubated in a cup with a mesh bottom and placed over an air bubbler. Fish were kept at 60°F with an even light cycle (12L: 12D) for the entirety of the experiment. All families were kept on one of two recirculating flow-through water racks, which consisted of a series of particular, biological, and IV filters and had three different shelves (Aquaneering, San Diego, CA). We replaced 10% of each tank’s water each day. Family tank position was pseudorandomly assigned so that all populations were evenly distributed across both racks and the three levels of shelves. Importantly, we elected to rear the fish and measure their behavior in freshwater (<5‰), thereby simulating the conditions that marine sticklebacks encounter when they move into freshwater. Because the marine populations studied here are naturally anadromous (Rabbit Slough [M; Bell 2016]; Resurrection Bay [M; R. King, personal communication]) and spawn in fresh/brackish waters, their early offspring development typically occurs under low salinity.

Behavioral Assays

Summary of the Behavioral Assays. Neophilia was measured as response to a novel object. Boldness was measured as latency to emerge from a refuge, a reliable and widely used behavioral assay in fishes (Wilson and Godin 2009; Pearish et al. 2013). Boldness was quantified at the individual level as average latency to emerge across trials. Behavioral flexibility was measured in a barrier task: after pretraining individuals to expect a food reward upon emergence from a refuge, individuals were confronted by a transparent barrier that they had to swim around to get the food reward. Individuals that continue to follow the search pattern established during training spend relatively more time at the point of the barrier closest to the food reward (“barrier apex”), which we interpret as relatively low flexibility. In contrast, individuals that quickly break away from the previously established behavior pattern spend relatively little time at the apex of the barrier, which we interpret as relatively high flexibility. A previous study in sticklebacks found that time at the apex of the barrier predicts reversal learning performance (Bensky and Bell 2020), another common metric of behavioral flexibility.

Experimental Methods. For full detailed methods, see “Supplementary Methods” in the supplemental PDF. We used 18 observation tanks (36 cm L × 33 cm W × 24 cm H) for behavioral testing in blocks of 18; trials were video recorded, and behavior was coded by M. K. Bensky. When the fish were approximately 8 months of age (approximately 40 mm standard length), the testing phase of the experiment began. Behavioral assays were carried out over the course of 5 months. Families, sexes, and populations were measured such that male and female offspring from different families and from different populations were measured in the same block. Individuals were randomly selected from each family, and their weight and length were measured. During the testing phase of the experiment, fish were fed only as part of the behavioral tests to help maintain motivation.

Acclimation Phase

To ensure that an individual had acclimated to its home tank and was motivated to eat during the behavioral tests, it was presented with food via a petri dish at the center of its home tank, and the individual had to eat the food within 10 min on three consecutive days to proceed to the next step. On average, it took 5.1 days for fish to meet this criterion (range = 3–17).

Novel Object Test (Neophilia)

An individual’s response to a novel object (toy lion; 10 cm L × 7 cm H; Terra by Battat, Montreal) was recorded once, the day after the fish met the criterion in the acclimation phase. The toy lion was selected as a novel object because the fish had no prior experience with this object, there was no presumed evolutionary history with the object’s shape, and it did not contain the color red, which elicits a strong behavioral response in sticklebacks (Tinbergen 1951).

For the 5 min that followed an individual’s initial approach of the object, we recorded how much time the individual spent orienting toward the novel object while it was within one body length of the object. We interpret more time spent near and oriented toward the novel object as greater time investigating the object (i.e., higher neophilia).
Latency to Emerge (Boldness)

For each individual, we recorded its latency to emerge from a refuge on its first four training trials for the barrier task (described below) and used the average of those measures as a proxy for boldness (video 1). The refuge was an opaque, cylindrical PVC pipe with caps on both ends (10 cm in diameter, 10 cm height) and a 32-mm-diameter hole on the side that was blocked with a removable cork. The refuge was novel to the fish on the first trial. Food was present during the emergence test. However, the acclimation phase of the experiment ensured that fish went into this stage with comparable food motivation. Additionally, once a fish began the experiment, it was fed only during the trials, and during the emergence trials, all of the individuals consistently ate, which suggests that they were similarly motivated during the emergence test. Latency to emerge was repeatable across the four training trials within each population (r = 0.504–0.738; table S2).

Barrier Detour Task (Flexibility)

Pretraining for the barrier detour task started on the same day the novel object test was completed. The goals of pretraining were to train the individual to learn that there would consistently be a food reward in the middle of pretraining were to train the individual to learn that the food reward, and gently pour the fish out of the shelter if necessary.

Training for the barrier task was criterion based. To move on to the barrier task following pretraining, the individual had to emerge from the shelter within 10 min and directly approach and eat the food reward within 5 s on three of the four trials. The one failed attempt could not be on the fourth trial; this requirement ensured that the fish would be motivated throughout the four trials. Fish were given a maximum of 4 days to reach the criterion. Fifteen fish did not meet the criterion (Big Beaver [E]: 7; Cornelius [E]: 1; Loberg [N]: 1; Cheney [N]: 3; Rabbit Slough [M]: 2; Resurrection Bay [M]: 1). Within Big Beaver, four of the seven individuals were from the same family, and two of the seven were from a different family.

Once an individual met the criterion, the individual moved on to the barrier detour task the following day. This task also comprised four trials. The first two trials were exactly the same as the pretraining trials to reinforce the direct search pattern. On the third trial a transparent, semicircular barrier was placed between the shelter and the food reward. The opening into the barrier area was positioned directly in front of the entrance to the shelter. After removing the cork the individual was allowed 30 min to emerge from the shelter, navigate around the barrier, and eat the food reward (video 2). The observer recorded the duration of the first bout (no break in contact longer than 5 s) at the apex of the barrier. To confirm that fish that spent little time at the barrier apex during the third trial were still motivated to eat, their behavior was observed for a fourth trial during which no barrier was present.

Altogether, a total of n = 262 individuals from n = 8–11 families per population (n = 2–7 full sibs per family) completed the novel object test and boldness assay. A total of n = 247 individuals from n = 8–11 families per population (n = 1–4 full sibs per family) completed those two tasks and the barrier task. The experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois Urbana-Champaign (IACUC protocol 15077).

Statistical Analysis

R version 3.5.3 (http://www.r-project.org/) was used for statistical analyses. Positively skewed variables were log transformed to improve normality, and model residuals were also visually inspected for deviations from normality.

We used linear mixed models (R Core Team 2013; package lme4, function lmert) to examine the behavioral data in each assay separately. We created models in which population and sex were included as fixed factors and body length was included as a covariate. Family ID was included as a random variable. The statistical significance of the effect of family ID was assessed via log-likelihood ratio test (i.e., by comparing models with and without the effect of family ID; R Core Team 2013; package care, function anova). We infer that there is suggestive evidence for an underlying heritable basis to a trait when it differs among populations (because they were reared in a common garden) and/or when family ID improves model fit.

After confirming that there was indeed significant variation among populations in the behavioral traits, we then tested our a priori hypothesis that marine and well-established freshwater populations differed in each trait by creating a contrast matrix that directly compared these two population types (R Core Team 2013; package multcomp,
function glht). Post hoc contrasts then focused on the two newly derived populations (Cheney Lake [N] and Loberg Lake [N]) because visual inspection of the data suggested that they are different and we were interested in the rate of rapid adaptation in these newly derived populations. The first post hoc contrast was between Cheney Lake (N) and Loberg Lake (N). Then Cheney Lake (N), the newest freshwater population (8 years from collection), was compared with the established freshwater populations, while Loberg Lake (N; 28–34 years from collection) was compared with the marine fish. Contrast matrices were constructed so that post hoc P values were adjusted for multiple comparisons.

To explore the heritable basis of these traits, we computed broad-sense heritabilities and genetic correlations within each population following Hadfield (2010) and Wilson et al. (2010). For all models, we included individual ID as a random effect relating individuals to their additive genetic values through a pedigree data set. Given the effect of body length on neophilia, length was included as a fixed factor in any model that included this behavioral measure. We used weakly informative inverse gamma priors for the residual and genetic effects (by setting the MCMCglmm parameters $V = 1, \nu = 0.002$). To test for genetic correlations, a $2 \times 2$ covariance matrix was specified with the degree of belief parameter set to $n = 1.002$. Behavioral measures were log transformed for this analysis, and a Gaussian distribution was used. The posterior distribution was sampled every 3,000 times (thinning interval), following a burn-in period of 100,000 iterations with a total run of 10,000,000 iterations. The relatively modest number of families and full sibs within each family resulted in large confidence intervals around the estimates; therefore, we interpret those results with some caution.

To examine the structure of the phenotypic variation both among and within populations, we examined correlations between traits at the individual, family, and population levels. All of the data have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3j9kd51hg; Bensky and Bell 2022).

Results

Sticklebacks from Dispersing Populations Were Bold

There was significant variation among populations in boldness (latency to emerge from a refuge; $F_{5,59} = 3.30, P = .01$; table S3; fig. 1). Sticklebacks from dispersing populations (marine) were bolder than fish from well-established freshwater populations ($z = 2.745, n = 262, P = .006$; table S4, top). The two recently derived populations provide insight into the rate at which boldness diverged from the ancestral marine behavioral type. Sticklebacks from Cheney Lake (N), a population that was established 8 years before collection, resembled marine

![Figure 1: Variation among populations in boldness (latency to emerge from a refuge). Smaller values indicate greater boldness. Boxplots with individual data points superimposed are shown. Marine populations are indicated by M and shades of red. Newly derived populations are indicated by N and shades of purple. Well-established freshwater populations are indicated by E and shades of blue.](image-url)
populations and differed from the well-established freshwater populations (Cheney [N] vs. well-established: \( z = -2.497, n = 262, P = .034 \); table S4, bottom). In contrast, sticklebacks from Loberg Lake (N), a population that was established 28–34 years before collection, more closely resembled the well-established freshwater populations (Loberg Lake [N] vs. marine: \( z = 2.429, n = 262, P = .041 \). After correcting for multiple comparisons, there was a trend for sticklebacks from Cheney Lake (N) to emerge faster compared with sticklebacks from Loberg Lake (N; \( z = -2.249, n = 91, P = .065 \)).

There was significant variation in boldness among families within populations (family ID improved model fit; \( \chi^2 = 9.394, df = 1, P = .002 \). Estimates of broad-sense heritability of boldness in the six populations ranged from 0.095 to 0.766 (average \( \bar{h}^2 = 0.461 \) and was significantly different from zero in the Big Beaver (E) and Cornelius (E) populations (table S5).

### Sticklebacks from Established Populations Were Flexible

There was significant variation among populations in behavioral flexibility (time at the apex of a barrier; \( F_{5,138} = 3.495, P = .008 \); table S3; fig. 2). Sticklebacks from the well-established freshwater populations were more flexible (spent less time at the apex of a barrier) compared with sticklebacks from the marine populations (\( z = -2.175, n = 158, P = .030 \); table S4, top). As was the case for boldness, the two recently derived populations show different patterns: flexibility in Cheney Lake (N), the most recently established freshwater population, resembled flexibility in the dispersing populations, while flexibility in Loberg Lake (N), the freshwater population that was established 28–34 years before collection, resembled flexibility in the well-established freshwater populations (Cheney Lake [N] vs. Loberg Lake [N]: \( z = 2.821, n = 87, P = .013 \); Cheney Lake [N] vs. well established: \( z = 3.373, n = 131, P = .002 \); table S4, bottom).

There was significant variation among families within populations in flexibility (\( \chi^2 = 7.350, df = 1, P = .007 \). Estimates of broad-sense heritability of flexibility in the six populations ranged from 0.156 to 0.829 (average = 0.418) and was significantly different from zero in the Cornelius (E) population (table S5).

### Smaller Fish Were More Neophilic

Neophilia (time near and oriented to a novel object) did not vary among populations or between the sexes (table S3) and did not vary in a systematic manner among the different types of populations (fig. 3). Larger fish were less neophilic (\( \beta = -0.033, t = -2.631, df = 247.79, P = .009 \); fig. S2; table S3). We did not detect variation among families within populations in neophilia, as family ID did not significantly improve model fit (\( \chi^2 = 2.369, \)

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**Figure 2:** Variation among populations in flexibility (time at the apex of the barrier). Smaller values indicate greater flexibility (i.e., less time persisting on a previously successful behavior pattern). Boxplots with individual data points superimposed are shown. Marine populations are indicated by M and shades of red. Newly derived populations are indicated by N and shades of purple. Well-established freshwater populations are indicated by E and shades of blue.
Estimates of broad-sense heritability of neophilia in the six populations ranged from 0.067 to 0.861 (average 0.291) and was significantly different from zero in the Cornelius (E) population (table S5).

Sticklebacks Consistently Show a Boldness-Flexibility Syndrome

Given that sticklebacks from the marine populations were bolder and less flexible compared with sticklebacks from newly derived populations, we tested how boldness and flexibility were correlated with one another. Consistent with the pattern described above, individuals that were bolder (quickly emerged from the refuge) were less flexible (spent more time at the barrier apex; \( r = -0.522, n = 245, P < .001; \) table S6), and this pattern was also evident at the family \( (r = -0.688, n = 62, P < .001) \) and population \( (r = -0.91, n = 6, P = .01) \) levels (fig. 4). The average genetic correlation between boldness and flexibility within the six populations was \( r = -0.642 \) (range: \(-0.467 \) to \(-0.745\)) and was significantly

Figure 3: Variation among populations in neophilia (time near and oriented toward the novel object). Boxplots with individual data points superimposed are shown. Marine populations are indicated by M and shades of red. Newly derived populations are indicated by N and shades of purple. Well-established freshwater populations are indicated by E and shades of blue.

Figure 4: Relationship between flexibility (time at the barrier apex) and boldness (latency to emerge) within and among populations. Marine fish are relatively bold (emerge quickly), while flexibility (spending less time at the barrier apex) increases as freshwater populations become increasingly established. Large circles show population means, medium circles show family means, and small circles show individual data points. Marine populations are indicated by M and shades of red. Newly derived populations are indicated by N and shades of purple. Well-established freshwater populations are indicated by E and shades of blue.
different from zero in the Cornelius (E) population (table S5).

Discussion

The possibility that behavioral and cognitive traits might facilitate and evolve during natural biological invasions is intriguing but difficult to study directly. Here, we took advantage of a model system for invasions to test the hypothesis that boldness is favored in dispersers and that neophilia and flexibility are favored in populations that are becoming established. We found that boldness and flexibility differed in a systematic way between marine populations and freshwater populations that differed in time since establishment. Specifically, the marine populations were bold, while the well-established freshwater populations were flexible. These traits varied between the two new freshwater populations relative to time since establishment. This pattern supports the hypothesis that these traits evolved in a way that facilitated successful colonization. A valid concern is that time since establishment might be confounded with other ecological factors that could be driving the phenotypic differences between newly derived and well-established populations. It is unlikely that two of the most important factors known to drive divergence among stickleback populations—predation risk and lake depth—are responsible for the differences because all of the freshwater populations are subject to predation by salmonids, piscivorous birds, and invertebrate predators and lake attributes do not systematically vary with time since establishment (table S1). That being said, the very presence of sticklebacks in these lakes could change the ecology via eco-evolutionary feedbacks on the community and ecosystem (e.g., Brunner et al. 2017), which is an interesting challenge for future work.

Boldness and flexibility were negatively correlated at the individual, family, and population levels, broadly consistent with coping styles theory (Koolhaas et al. 1999) and with the idea of cognitive styles (Sih and Del Giudice 2012). Differences in boldness and flexibility were evident in a common-garden environment, and there was significant variation among families in both traits; therefore, their divergence might be evolutionary in origin. If boldness is favored in invaders during the initial dispersal stage while flexibility is favored during the establishment stage, then a tight link between boldness and flexibility could facilitate success during both the dispersal stage and the establishment stage and therefore play a key role in facilitating invasion success in this species (Sih et al. 2012). Selection favoring boldness in dispersers and flexibility in immigrants could cause the population to move along the line defining the correlation between boldness and flexibility whenever sticklebacks diverge from the ancestral marine behavioral type (fig. 4). An outstanding question is what maintains the extensive phenotypic and genetic variation in boldness and flexibility within the populations (figs. 1–4). Selection might not be strong enough to effectively purge the variation, and/or there may be ongoing gene flow between marine and freshwater habitats that maintains alleles associated with behavioral flexibility at low frequency in marine populations (transporter hypothesis; Schluter and Conte 2009).

We found evidence for parallelism in the two marine populations and the two well-established populations (i.e., the two marine populations were bold, while the two well-established populations were flexible). In contrast, the two newly derived populations (Cheney Lake [N] and Loberg Lake [N]) were different from each other: Cheney Lake resembled the marine populations, while Loberg Lake resembled the well-established populations. There are at least two potential explanations for these results. First, the pattern might reflect differences in time since establishment. Cheney Lake (N) was founded more recently than Loberg Lake (N; 9 vs. 28–34 years before this study); perhaps it takes longer than 10 generations for behavioral and cognitive traits to diverge from the ancestral marine behavioral type. Alternatively, or in addition, the phenotypic differences between sticklebacks from Loberg Lake (N) and Cheney Lake (N) could reflect differences in the way that the two lakes were colonized; Loberg Lake (N) was naturally colonized, while sticklebacks were experimentally introduced to Cheney Lake (N). If particularly flexible individuals were more likely to disperse into Loberg Lake (N) but a random sample of behavioral types were artificially introduced into Cheney Lake (N; Cote et al. 2010; Edelaar and Bolnick 2012; Canestrelli et al. 2016), then nonrandom dispersal could be contributing to the rapid evolution of increased flexibility in Loberg Lake (N). However, the fish that were originally introduced into Cheney Lake (N) were caught by minnow traps in Rabbit Slough (M; Bell 2016), and if bold fish are more likely to enter traps (Wilson et al. 1993), then Cheney Lake (N) might actually have been founded by particularly bold individuals. Together with evidence from the literature (Cote et al. 2010; Canestrelli et al. 2016) and our findings that boldness and flexibility are tightly negatively correlated with one another, the explanation that nonrandom dispersal is what has mainly contributed to the population differences in flexibility seems unlikely. Further studies tracking how behavioral and cognitive traits change over time in the Cheney Lake (N) population (and similar experimental lakes; e.g., Scout Lake) could help discriminate between the time since establishment and nonrandom dispersal hypotheses.

We originally hypothesized that neophilia would be favored in derived populations because seeking and/or
being willing to investigate novel stimuli may help individuals in newly establishing populations locate new habitats and discover novel resources, but we found no support for this hypothesis. One possible explanation for the failure to find systematic differences in neophilia among the populations is that neophilia may actually be disadvantageous in a new environment because it can expose animals to dangerous stimuli they have never encountered before. Another potential (nonexclusive) explanation based on our results is that neophilia may not evolve as readily because it may be less heritable (effect of family ID was nonsignificant, lower $H^2$ estimate). Instead, neophilia may be more influenced by age or experience: in this study, smaller (and younger: $r = -0.159, t = -2.595, n = 262, P = .01$) fish were more neophilic, which is consistent with other studies that have shown that novelty seeking decreases with age (Stansfield and Kirstein 2006).

Sticklebacks are a powerful model system for understanding how and why certain traits repeatedly evolve whenever organisms invade new habitats. Accumulating evidence suggests that sticklebacks have evolved mechanisms for rapidly adapting to new environments, with alleles conferring the freshwater-adapted phenotype maintained at low frequency in the ocean (Colosimo et al. 2005). But the role of behavioral traits such as boldness and cognitive traits such as flexibility in facilitating dispersal and divergence in this and other systems has received less attention (Foster et al. 2015). Behavioral and cognitive traits could play a special role in facilitating biological invasions and subsequent divergence because they influence how animals interact with their environments and are therefore at the front lines of adaptation. Our results suggest that a behavioral trait (boldness) plays an underappreciated role in facilitating the ability of marine sticklebacks to move into new habitats and that a cognitive trait (flexibility) could influence their success once they get there. Moreover, our results suggest that a behavioral mechanism—a behavioral syndrome linking boldness and flexibility together—may contribute to rapid adaptation in this and other organisms.

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Statement of Authorship

Both authors agree to submission of the manuscript. M.K.B. designed the study, collected and analyzed the data, and cowrote the manuscript. A.M.B. designed the study, contributed to interpretation of the results, and cowrote the manuscript. The authors do not have any conflicts of interest.

Data and Code Availability

Data have been archived in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3j9kd51hg; Bensky and Bell 2022). The code has been deposited in Zenodo (https://doi.org/10.5281/zenodo.6636245).

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