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Optimal outbreeding is shaped during larval life history in the splash pool copepod *Tigriopus californicus*

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Abstract

Inbreeding and outbreeding depression are dynamic forms of selection critical to mating system evolution and the efficacy of conservation biology. Most evidence on how the relative severity and timing of these forces are shaped is confined to self-fertilization, distant outcrossing, and intermediate "optimal outcrossing" in hermaphrodites. We tested the notion that closed population demographics may reduce and delay the costs of inbreeding relative to distant outbreeding in an intertidal copepod with separate sexes and a biphasic larval/post-metamorphic life history (*Tigriopus californicus*). At three lifecycle stages (fecundity, metamorphosis, and post-metamorphosis), we quantified the effects of inbreeding and outbreeding in crosses with varying degrees of recent common ancestry. Although inbreeding and outbreeding depression have distinct genetic mechanisms, both manifested the same stage-specific consequences for fitness. Inbreeding and outbreeding depression were not apparent for fecundity, post-metamorphic survival, sex ratio, or the ability to acquire mates, but inbreeding between full siblings and outbreeding between interpopulation hybrids reduced the fraction of offspring that completed metamorphosis by 32% and 47%, respectively. On average, the effects of inbreeding on metamorphic rate were weaker and nearly twice as variable among families than those of outbreeding, suggesting genetic load was less pervasive than the incompatibilities accrued between divergent populations. Overall, our results indicate the transition from larval to juvenile life stages is markedly susceptible to both inbreeding and outbreeding depression in *T. californicus*. We suggest stage-specific selection acting concurrently with the timing of metamorphosis may be an instrumental factor in shaping reproductive optima in species with complex life histories.

Key words: inbreeding depression, metamorphosis, outbreeding depression, *Tigriopus californicus*

Introduction

The amount of common ancestry shared among mates and the resulting consequences for fitness are key attributes of conservation and evolutionary biology. On one end of the spectrum, inbreeding via self-fertilization or mating among close relatives increases homozygosity, exposes deleterious recessives to selection, and often reduces mean fitness through inbreeding depression (Charlesworth and Charlesworth 1999). On the other, crosses between species or distantly related conspecifics can also have costs, particularly when outbreeding dismantles coevolved or locally adapted gene combinations (Price and Waser 1979; Burton and Barreto 2012). Substantial progress has been made in the study of both inbreeding and outbreeding depression separately, but exactly how these forces operate together to shape reproductive success is not well understood. Selection against close inbreeding and distant outbreeding may broadly maintain reproductive optima so that mates with intermediate degrees of shared ancestry tend to have the greatest fitness (Shields 1982; Knowlton and Jackson 1993). Yet, nuances in the relative severity and timing of inbreeding and outbreeding depression are critical to understanding the evolution of mating systems (Goodwillie et al. 2005; Duthie and Reid 2016), as well as predicting risks in management strategies involving controlled breeding (Edmands 2007).

Reproductive optima shaped by inbreeding and outbreeding depression have received considerable attention in hermaphroditic plants where both self-fertilization and outcrossing between distant conspecifics can have negative effects on seed set, seedling survival, and flowering (Price and Waser 1979; Waser and Price 1989). Studies evaluating "optimal outcrossing" have expanded across hermaphroditic plants and animals to address differences in demographics, environment, ploidy, and life history (Billingham et al. 2007; Dolgin et al. 2007; Escobar et al. 2008; Grindeland 2008; Stojanova et al. 2021). Generally, populations and species with closed demographics and a history of self-fertilization are more likely to purge genetic load and gain a reprieve from inbreeding depression (Lande and Schemske 1985; Schemske and Lande 1985). Moreover, self-seeding population dynamics lend themselves

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to fine-scale adaptation and the coevolution of interacting genetic elements, making lineages with closed demographics more susceptible to outbreeding depression when these forces are dismantled (Schmitt and Gamble 1990; Edmands and Timmerman 2003).

Not only are the relative severities of inbreeding and outbreeding depression linked to demographic history, but the timing of selection and the lifecycle stage in which they are manifested is predicted to be as well (Lesaffre and Billiard 2020). In a landmark review, Husband and Schemske (1996) detailed the correlated evolution of self-fertilization and the stage-specific consequences of inbreeding depression in predominantly selfing and outcrossing plants. The authors found that in species with a history of self-fertilization, inbreeding depression was purged from the early lifecycle stages of seed production, germination, and juvenile survival, but persisted in subsequent growth and reproduction defined as flower production, inflorescence number, and seed/flower ratio. In comparison, outcrossing species exhibited severe inbreeding depression early in the lifecycle via reduced fecundity and juvenile survival. These patterns are likely attributed to deleterious recessives with stage-specific effects and the ineffectiveness of selection to remove those expressed after maturation and reproductive age (Hamilton 1966). As a result, the purging of early-acting genetic load is predicted to delay inbreeding depression from fecundity and juvenile survival to later stages of the lifecycle in species practicing self-fertilization.

While associations between demographic history, mating systems, and the timing of selection are supported among self-compatible hermaphrodites, the extent to which these patterns apply more broadly is unclear. Models predict that genetic purging is unlikely to operate in the same fashion under biparental inbreeding and self-fertilization (Uyenoyama 1986; Duthie and Reid 2016; Porcher and Lande 2016). Rather, biparental inbreeding is predicted to increase homozygosity more gradually than self-fertilization, potentially making this form of inbreeding more efficient at removing deleterious recessives through differential selection (Duthie and Reid 2016; Porcher and Lande 2016). In theory, efficient purging of deleterious recessives via biparental inbreeding may effectively lessen the costs of inbreeding depression, especially for traits expressed early in the lifecycle. However, fewer works have detailed the relative effects of inbreeding and outbreeding depression within species with separate sexes (but see De Meester 1993; Aspi 2000; Peer and Taborsky 2007). As with self-fertilization, multiple generations of biparental inbreeding may reduce and delay inbreeding depression within closed populations, but whether or not both forms of inbreeding exhibit similar evolutionary dynamics remains a key question. Addressing how gradations of shared ancestry influence fitness under different demographic- and life histories may provide valuable insight into the factors shaping reproductive optima across species.

Estimates of the inbreeding coefficient (F_{IS}) suggest lineages with a history of inbreeding may be equally as common in terrestrial seed plants and some groups of marine invertebrates (Olsen et al. 2020). Absent or shortened dispersive stages, barriers to recruitment, and tolerance of inbreeding contribute to closed demographics in members of this group (Hellberg 1996; Burton 1997; Sanford and Kelly 2011; Olsen et al. 2016, 2021; Burgess et al. 2023; Olsen and Levitan 2023). Specifically, the harpacticoid copepod *Tigriopus californicus* exhibits many characteristics indicative of closed demographics, including fine-scale genetic differentiation, local adaptation, heterosis among the F_1 offspring of some interpopulation crosses, and outbreeding depression in F_2 interpopulation hybrids (Burton 1986, 1990; Edmands 1999; Ellison and Burton 2008; Pereira et al. 2017). Hybrid vigor in the first generation of interpopulation crosses, likely due to increased heterozygosity and the masking of deleterious recessives, has the potential to shift the reproductive optimum toward outbreeding in this species. However, multiple genomic evaluations detail the disproportionate role of incompatibilities between the mitochondrial and nuclear genomes in the inviability and phenotypic breakdown of F_2 interpopulation hybrids (Barreto et al. 2018; Lima et al. 2019; Healy and Burton 2020; Han and Barreto 2021).

Given that the genetic bases of inbreeding and outbreeding depression appear distinct in *T. californicus*, in that the costs of inbreeding are often tied to deleterious recessives while those of distant outbreeding are driven by epistatic incompatibilities involving both nuclear–nuclear and mito–nuclear interactions, the severities and timing of these forces may also be different. Closed demographics, a history of biparental inbreeding, and the potential purging of early-acting deleterious recessives may weaken and delay inbreeding depression relative to outbreeding depression. Yet, less emphasis has been placed on the consequences of inbreeding in *T. californicus* (but see Brown 1991; Palmer and Edmands 2000), making this an appropriate system to contrast the effects of inbreeding and outbreeding depression across the lifecycle. Here, we evaluate the relative severity, variability, and timing of these forces within and between two populations encompassing much of the species widespread range along the west coast of North America. We interpret our findings through two broad predictions for species with closed demographics and a history of inbreeding: 1) the costs of inbreeding are predicted to be less severe than those of outbreeding, and 2) the timing of inbreeding depression is predicted to manifest in later stages of the lifecycle compared with outbreeding depression.

Methods

Study species

The lifecycle of *T. californicus* consists of 12 stages—six naupliar larval stages and six copepodid stages, the sixth being the adult. Adult males clasp immature females (copepodid stages II–V) with their antennae and "guard" their future mate until she reaches maturity when she is inseminated and released. Females only mate once, but males can mate numerous times; in the lab, a single male successfully fertilized four females in 72 h (Burton 1985). In natural populations, the number of mates fertilized by a single male is limited by the need to guard individual females from competing males, an activity that typically lasts 1 to 5 d depending on the developmental stage of the clasped female. Median longevity of both males and females is strongly impacted by temperature, averaging approximately 50 d at 25 °C and 150 d at 15 °C (Watson et al. 2022). Assessments of mate choice in the laboratory indicate individuals avoid full siblings when given the option of an unrelated mate (Palmer and Edmands 2000), which may limit the historical effectiveness of purging. However, individuals willingly mate with full siblings when this option is not available, and the potential for inbreeding and purging due to background shared ancestry, restricted dispersal, and genetic subdivision is considerable in this species.

Sampling and breeding design

T. californicus were collected from intertidal rocky pools in San Diego, CA (SD; 32.45, –117.25) and Strawberry Hill, OR (SH; 44.25, –124.11), United States. Copepods evaluated in this study were descendants of multiple collection events conducted in 2019 to 2022 from multiple pools in an approximately 10 × 10 m area within both sites. Collections were maintained in mass subcultures (11 SD and 13 SH subcultures) each comprised of hundreds to thousands of individuals. Copepods in culture were maintained in 400 mL of 35 ppt seawater at 20 °C with a 12-h light/dark cycle and fed dry spirulina, ground commercial fish food, and natural algal growth. Individuals were transferred regularly between subcultures of the same population in an effort to maintain genetic diversity and homogeneity among cultures until breeding experiments were conducted in 2022 and 2023.

We evaluated the consequences of inbreeding and outbreeding in SD and SH *T. californicus* with five experimental cross types: 1) full sibling, 2) paternal half-sibling, 3) unrelated intrapopulation, 4) reciprocal interpopulation (SD♂ × SH♀ and SH♂ × SD♀), and 5) reciprocal $F_1 \times F_1$ interpopulation crosses (SD♂SH♀ and SH♂SD♀). Crosses were evaluated in 48 replicate familial lines (26 SD and 22 SH), initiated by sampling gravid females from culture and isolating their offspring in 6-well plates. We leveraged the polygynous reproductive system of *T. californicus* and two generations of controlled breeding to establish individuals with varying degrees of recent common ancestry. We established paternal half-siblings by crossing male copepods from each replicate line with two virgin females from distinct cultures of the same population. Males from each line were also crossed with a female from the alternative population to produce F_1 interpopulation hybrids. In the subsequent generation, separate males from each replicate line were crossed with female full siblings, paternal half-siblings, and females from distinct cultures of the same population to limit any shared ancestry in unrelated intrapopulation crosses. As many combinations of $F_1 \times F_1$ interpopulation crosses among replicate lines were conducted as possible while avoiding inbreeding $F_1 \times F_1$ crosses within replicate lines. The fitness of each experimental cross type was quantified in the resulting offspring.

Estimates of absolute fitness across life-history stages

We evaluated four stage-specific assessments of absolute fitness in the offspring of experimental crosses: 1) number of larval offspring produced, 2) proportion metamorphosed, 3) post-metamorphic survival, and 4) cumulative fitness measured as the total number of adults generated from a single clutch. Females gravid from experimental crosses were placed individually in 6-well plates until their larval offspring hatched. On the day of hatching, the maternal copepod was removed, and the number of larval offspring in a single clutch was counted as an assessment of fecundity. We evaluated fecundity in the female's first clutch (as in Edmands 1999; Ellison and Burton 2008) and the mean fecundity across multiple subsequent clutches in separate analyses. Separate analyses were conducted on fecundity to facilitate comparison across studies that specifically evaluated the first clutch of experimental females. Larval offspring from a single clutch were placed in a petri dish in 40 mL of filtered seawater, fed dry spirulina, and incubated at 20 °C. The proportion of larvae that metamorphosed into copepodids (i.e. post-metamorphic juvenile copepods) was evaluated 14 d post-hatching (as in Edmands 1999; Ellison and Burton 2008). Only those clutches with at least 10 offspring were used in assessments of metamorphosis. Maternal copepods with clutches containing fewer than 10 offspring were retested to assess metamorphosis in subsequent clutches.

Following 28 d post-hatching, the number and sex of adult copepods were assayed to evaluate the proportion of post-metamorphic juveniles that reached maturity. We evaluated post-metamorphic survival in clutches containing as few as four juveniles. Additional analyses of post-metamorphic survival with a minimum of 10 juveniles, but fewer replicates, were also conducted. The total number of adult copepods produced in each clutch was assessed 28 d post-hatching as an estimate of cumulative fitness among experimental cross types.

Relative fitness estimates of inbreeding and outbreeding depression

We quantified inbreeding and outbreeding depression via relative fitness with the approach of Ågren and Schemske (1993). This approach measures the "relative performance" (RP) of inbred and outbred offspring compared with crosses of interest. The index ranges from –1 to 1, where positive values indicate inbreeding depression and negative values indicate outbreeding depression. The index gives equal weight to inbreeding and outbreeding depression and incorporates instances where inbred and outbred crosses exhibit greater fitness than the reference which can be important to quantifying the variability associated with inbreeding and outbreeding depression. We quantified inbreeding depression in full sibling crosses relative to intrapopulation crosses and outbreeding depression in $F_1 \times F_1$ crosses relative to interpopulation crosses with the equations:

$$\mathrm{RP}_{\text{inbreeding depression}} = 1 - \frac{w_{\text{full sibling}}}{w_{\text{intrapopulation}}}$$

when $w_{\text{full sibling}} \leq w_{\text{intrapopulation}}$

$$\mathrm{RP}_{\text{outbreeding depression}} = \frac{w_{\text{F1} \times \text{F1}}}{w_{\text{interpopulation}}} - 1$$

when $w_{\text{F1} \times \text{F1}} \leq w_{\text{interpopulation}}$

In instances where the inbred or outbred cross had greater fitness than the reference, the following equations were used to maintain the –1 to 1 scale:

$$\mathrm{RP} = \frac{w_{\text{intrapopulation}}}{w_{\text{full sibling}}} - 1$$

when $w_{\text{full sibling}} > w_{\text{intrapopulation}}$

$$\mathrm{RP} = 1 - \frac{w_{\text{interpopulation}}}{w_{\text{F} \times \text{F1}}}$$

when $w_{\text{F1} \times \text{F1}} > w_{\text{interpopulation}}$.

We elected to quantify outbreeding depression among $F_1 \times F_1$ interpopulation crosses relative to interpopulation crosses, as opposed to intrapopulation crosses, in order to retain as many replicate familial lines in the analysis as possible. Both inbreeding and outbreeding depression were measured relative to corresponding crosses of the same replicate familial line.

Mating trials

We tested the ability of inbred and outbred male copepods to acquire female mates in competitive trials. We used the mate-guarding behavior of *T. californicus* to test if inbred and outbred males were less likely to acquire female mates compared with males from culture. Differences in female acquisition could be due to male–male competition and/or female choice. A similar approach was previously used to evaluate mate choice in *T. californicus* (Palmer and Edmands 2000). Following 28 d post-hatching, inbred males produced from full sibling crosses, "normal" males from unrelated intrapopulation crosses, and F_2 outbred males from $F_1 \times F_1$ interpopulation crosses were placed individually in 6-well plates and paired with a single similarly sized male selected from culture. Experimental and culture males were visually distinguishable due to differences in diet-based coloration. Inbred, "normal," and F_2 outbred males from experimental treatments become more transparent when fed only spirulina, whereas males in culture feed on natural algal growth and retain red coloration. Because our interest was in comparing the competitive ability of inbred, "normal," and outbred males, all of which were from experimental treatments, diet was not a confounding factor. Both males were acclimated for 15 min, after which a single virgin female was placed into the well. After 4 h, the trial was assessed to determine which of the two males successfully clasped the female.

Statistical analyses

Analyses in this study focused on comparing measures of fitness among experimental cross types and quantifying relative fitness within replicate lines in both SD and SH. When multiple clutches of the same experimental cross type and replicate line were assayed, we analyzed their mean estimates of fitness. Fecundity and proportional measures of fitness often could not be transformed to meet the assumptions of parametric testing. Instead, we used aligned rank transformed nonparametric mixed-effects models in the R (version 4.1.2) package "ARTool" to test for significant differences among cross types and to conduct post hoc multiple comparisons with Tukey's correction (Wobbrock et al. 2011; Elkin et al. 2021; R Core Team 2021). For each estimate of fitness, we treated the experimental cross type as a fixed factor with the replicate line as a random effect. Because the number of individuals varied among replicate crosses, we tested for density-dependent mortality in metamorphic and post-metamorphic assays of full sibling and $F_1 \times F_1$ interpopulation crosses with Thiel–Sen nonparametric linear regression in the R package "mblm" (Komsta 2013). Differences in the distributions of metamorphic rate among full sibling, unrelated intrapopulation, and $F_1 \times F_1$ crosses were evaluated by sampling with replacement from the original distributions with 10,000 bootstraps. In assessments of relative performance, inbreeding, and outbreeding depression were considered significant when their 95% confidence intervals did not overlap with zero. A 2 × 1 chi-square test was used to evaluate the ability of inbred and outbred males to acquire females compared with control males selected from culture.

Results

In total, we evaluated 117 crosses among *T. californicus* copepods with varying degrees of recent common ancestry ranging from full siblings to interpopulation hybrids. Analyses of fecundity showed no significant differences in the number of larval offspring produced from full sibling, paternal half-sibling, unrelated intrapopulation, reciprocal interpopulation, or reciprocal $F_1 \times F_1$ interpopulation crosses ($F = 1.88$, df = 4, $P = 0.12$; Fig. 1A). In a subsetted analysis, we detected a marginal effect of cross type on first clutch fecundity ($F = 2.60$, df = 4, $P = 0.05$), but neither full sibling nor $F_1 \times F_1$ interpopulation crosses were significantly different from intrapopulation crosses in these comparisons (Supplementary Fig. S1).

The proportion of larval offspring that completed metamorphosis was significantly reduced in both full sibling and $F_1 \times F_1$ interpopulation crosses compared with unrelated intrapopulation crosses ($F = 7.15$, df = 4, $P < 0.001$; Fig. 1B), and was not associated with initial larval density in full sibling (slope = −0.006, $P = 0.57$) or $F_1 \times F_1$ interpopulation crosses (slope < 0.001, $P = 0.95$). Resampled distributions of metamorphic rate indicated that while full sibling and reciprocal $F_1 \times F_1$ interpopulation crosses were not significantly different, the effects of inbreeding ($\bar{x} = 0.42$, $\sigma = 0.11$; Fig. 2) were nearly twice as variable among replicate familial lines as those of outbreeding ($\bar{x} = 0.27$, $\sigma = 0.06$; Fig. 2).

We detected significant differences among cross types in post-metamorphic survivorship ($F = 3.99$, df = 4, $P = 0.006$) that were not associated with initial juvenile density in full sibling (slope = 0.001, $P = 0.65$) or $F_1 \times F_1$ interpopulation crosses (slope < 0.001, $P = 0.99$). Post hoc comparisons with as few as four individuals per replicate indicated inbred copepods generated from full sibling crosses had lower post-metamorphic survival than those from interpopulation and $F_1 \times F_1$ interpopulation crosses. Nonetheless, the post-metamorphic survivorship of inbred copepods from full sibling crosses and outbred copepods from $F_1 \times F_1$ interpopulation crosses were not significantly different than those from unrelated intrapopulation crosses (Fig. 1C). In assessments with a minimum of 10 juveniles per replicate, the results were qualitatively the same, in that there was no significant difference in post-metamorphic survival between inbred or outbred copepods and those of unrelated intrapopulation crosses. Furthermore, there were no significant differences across experimental cross types in the relative abundance of males and females within clutches 4 wk after hatching ($F = 0.96$, df = 4, $P = 0.43$).

Inbreeding and outbreeding depression measured relative to crosses of the same familial line had stage-specific consequences in *T. californicus* (Fig. 3). Relative performance in the number of larval offspring hatched was not significantly different from zero in either full sibling (−0.13 ± 0.20; mean ± 95% CI) or $F_1 \times F_1$ interpopulation crosses (0.07 ± 0.25; Fig. 3A). We detected both significant inbreeding and outbreeding depression in the fraction of offspring that completed metamorphosis in full sibling (0.39 ± 0.26) and $F_1 \times F_1$ interpopulation (−0.37 ± 0.30) crosses (Fig. 3B). Neither

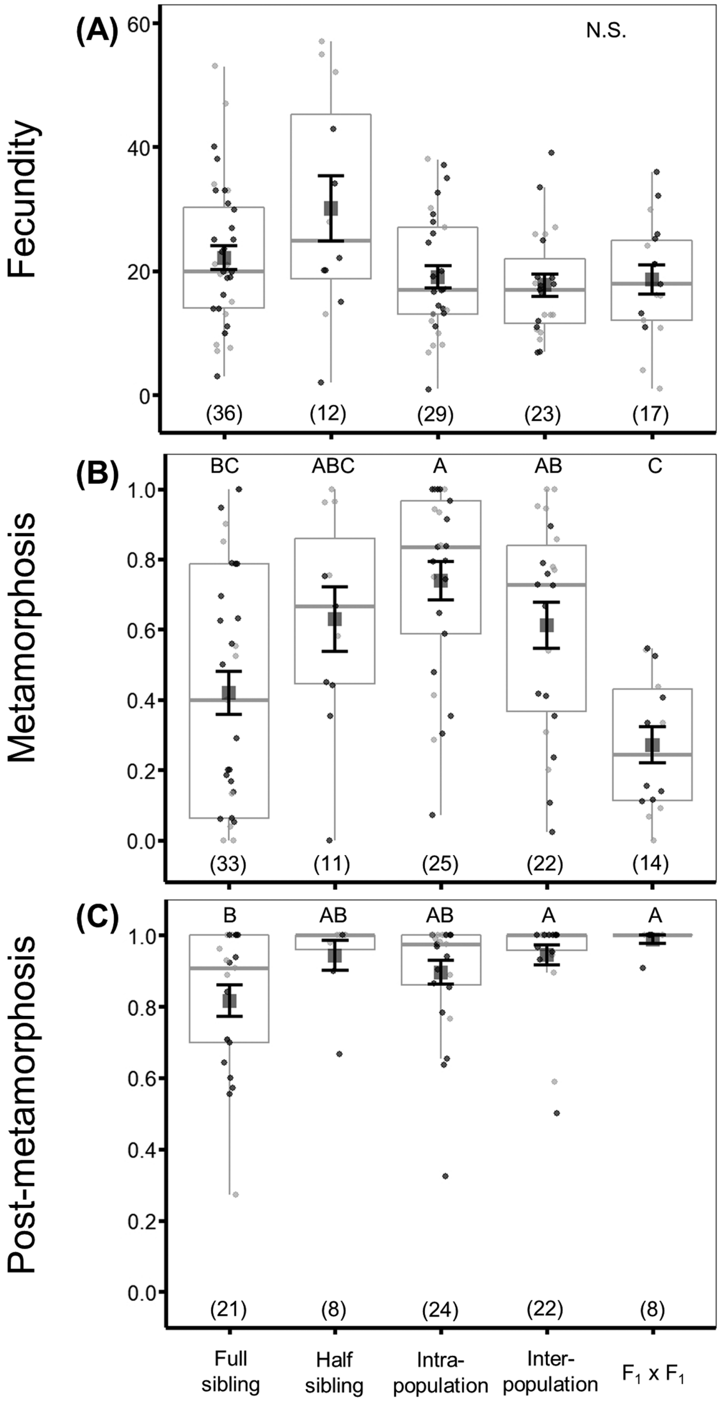

Fig. 1. Consequences of inbreeding and outbreeding across three life history stages of *Tigriopus californicus*. In A) fecundity, B) fraction metamorphosed, and C) post-metamorphic survival. Cross types include full sibling, paternal half-sibling, unrelated intrapopulation, reciprocal interpopulation, and reciprocal $F_1 \times F_1$ interpopulation crosses (SD♂ × SH♀ and SH♂ × SD♀). Within panels, the letters A, B, and C refer to the significance of between group comparisons. Black data points are SD-orientated crosses (i.e. within SD, SD♂ × SH♀, and $F_1 \times F_1$ SD♂SH♀); gray data points are SH-orientated crosses (i.e. within SH, SH♂ × SD♀, and $F_1 \times F_1$ SH♂SD♀). Boxes represent interquartile ranges, horizontal lines are medians, squares are means, and error bars are standard errors. Sample sizes in parentheses indicate the number of replicate familial lines evaluated in each cross type (Full sibling, Half sibling, Intrapopulation, and Interpopulation) and number of $F_1 \times F_1$ combinations.

inbreeding (0.03 ± 0.14) nor outbreeding (−0.01 ± 0.03) depression were detected in post-metamorphic survivorship from 2 to 4 wk of age (Fig. 3C).

Cumulative fitness measured 28 d post-hatching differed significantly among experimental cross types ($F = 7.10$, df = 3, $P < 0.001$). Inbreeding between full siblings and outbreeding between $F_1 \times F_1$ interpopulation hybrids resulted in fewer adult copepods compared with intrapopulation crosses at the 4-wk mark (Fig. 4). In 73% (16/22) of cases inbreeding between full siblings resulted in lower cumulative fitness than the intrapopulation cross of the same familial line (Supplementary Fig. S2). Similarly, in 78% (7/9) of familial

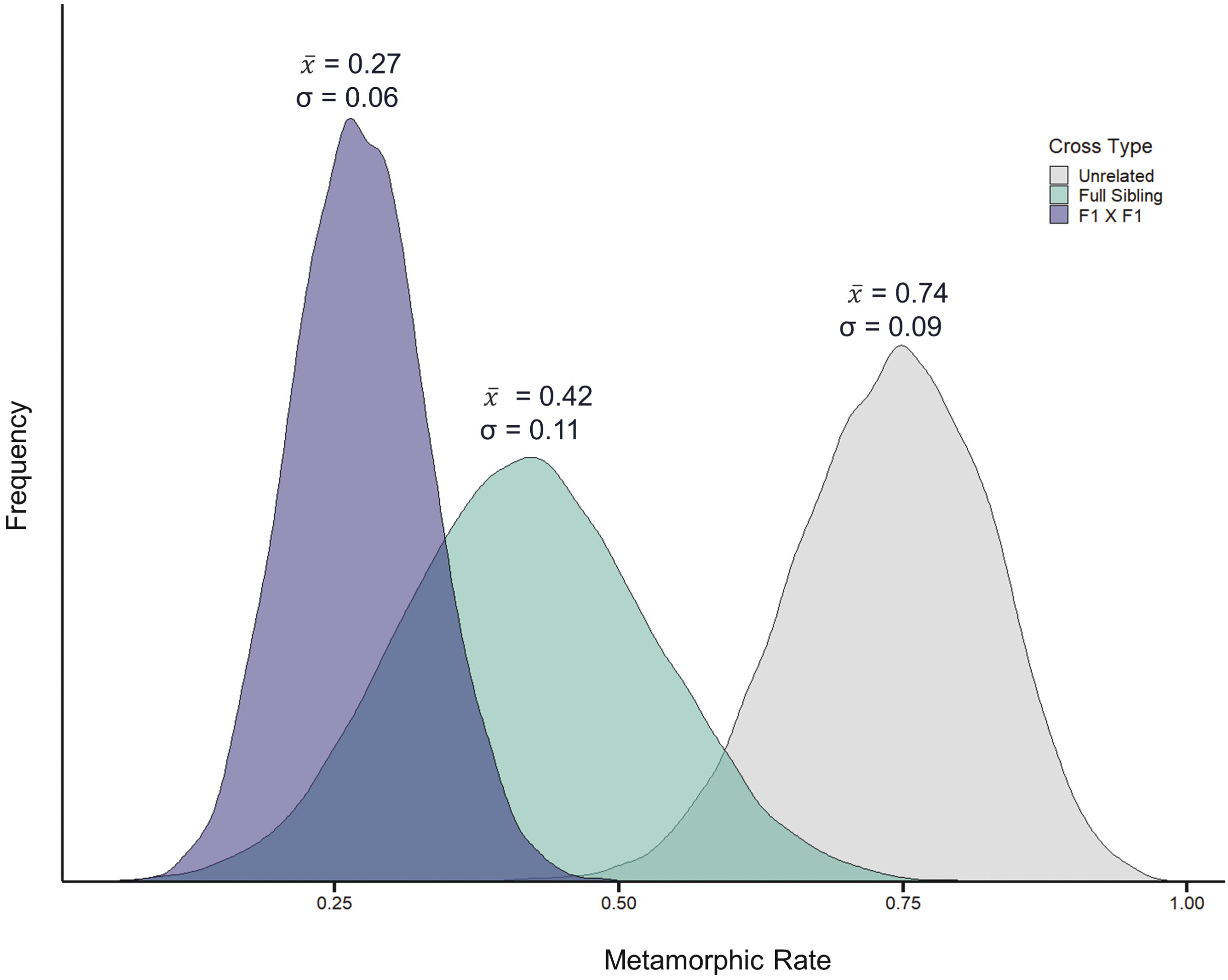

Fig. 2. Simulated distributions of metamorphic rate in *T. californicus* under unrelated intrapopulation, full sibling, and reciprocal $F_1 \times F_1$ interpopulation crosses. Distributions simulated by sampling with replacement from the original distributions with 10,000 bootstraps.

lines $F_1 \times F_1$ interpopulation crosses had reduced cumulative fitness compared with the corresponding interpopulation cross (Supplementary Fig. S2). In an assessment of adult vitality, we tested the ability of inbred and outbred males to acquire and mate-guard females in competitive trials (Table 1). Inbred males from full sibling crosses and outbred males from $F_1 \times F_1$ interpopulation crosses were equally as likely to clasp virgin females as control males in these assessments ($x^2 = 0.02$, df = 1, $P = 0.88$).

Discussion

Stage-specific mortality manifested during larval development and metamorphosis was a potent mechanism of both inbreeding and outbreeding depression in *T. californicus*. On average, 42%, 74%, and 27% of offspring from close inbreeding, random intrapopulation mating, and distant outbreeding, respectively, completed the metamorphic transition from larval to juvenile life stages. Our results support the notion that inbreeding may be less costly on average than distant outbreeding in species with closed population demographics. However, our findings do not support the prediction that inbreeding depression might be delayed to later stages of the lifecycle. Rather, they suggest the metamorphic process is critically susceptible to genetic or physiological elements expressed due to both close inbreeding and distant outbreeding in *T. californicus*. Given the complexity of transforming the body plan, we suggest metamorphosis may be uniquely prone to stage-specific selection and consequently an instrumental filter shaping reproductive optima in species with biphasic life histories.

Inbreeding and outbreeding depression did not affect fecundity, post-metamorphic survivorship, sex ratio, or male clasping ability in crosses within and between distant populations of *T. californicus*. In interpopulation crosses of this species, hybrid vigor is often expressed in the first generation followed by hybrid breakdown in subsequent generations (Burton 1990; Burton et al. 2006). We did not detect hybrid vigor in the F_1 offspring of reciprocal crosses between SD and SH. Instead, metamorphic success and cumulative fitness were greater on average among unrelated intrapopulation mates than in interpopulation crosses. The lower metamorphic rate among interpopulation crosses suggests our relative fitness estimates of outbreeding depression were lower for this stage than if intrapopulation crosses were used as a reference. Nonetheless, estimates of outbreeding depression during the metamorphic transition were significantly different from zero with the interpopulation cross as reference

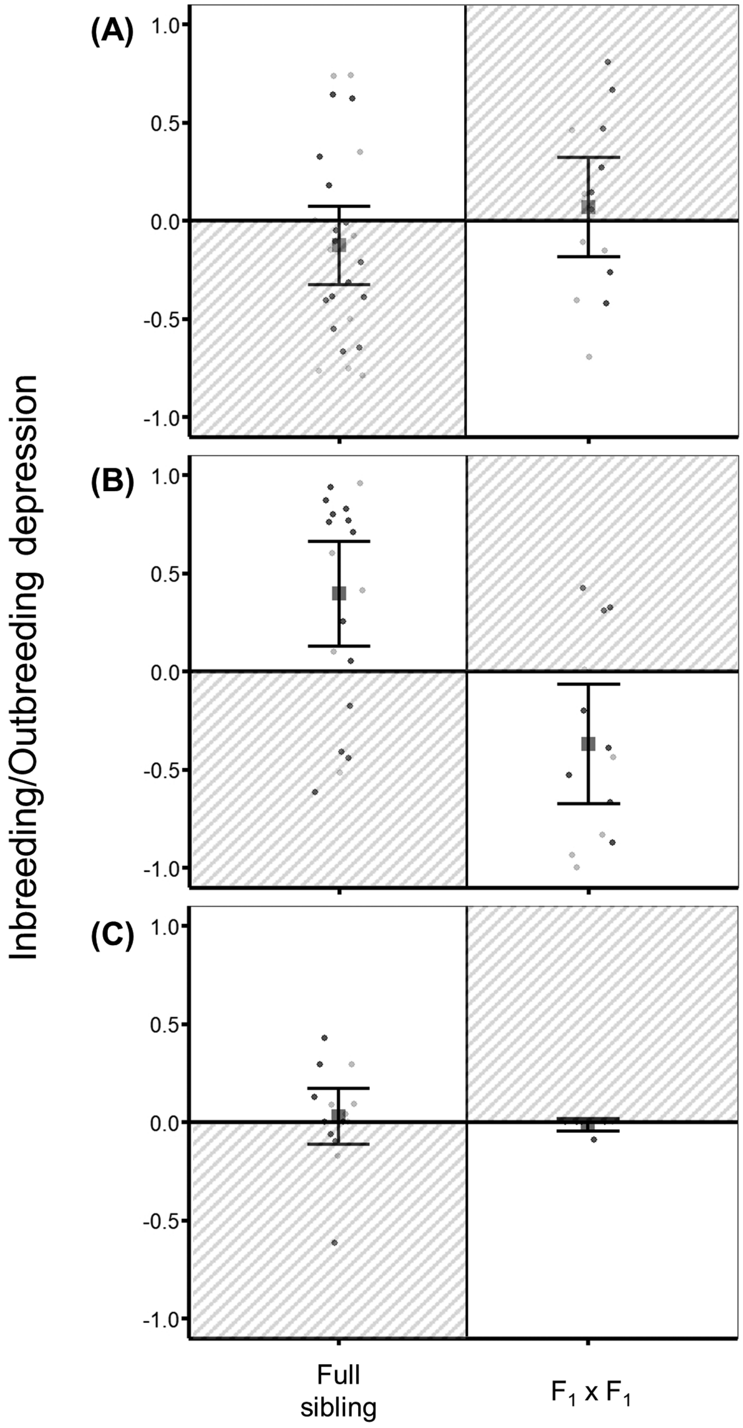

Fig. 3. Inbreeding and outbreeding depression across life history stages of *Tigriopus californicus*. In A) fecundity, B) fraction metamorphosed, and C) post-metamorphic survival. Positive values indicate inbreeding depression and negative values indicate outbreeding depression measured relative to corresponding crosses of the same replicate familial line. Hashed areas represent instances where inbred or outbred crosses had greater fitness than the reference cross. Black data points are SD-orientated crosses (i.e. SD full siblings, and F_1 × F_1 SD♂SH♀); gray data points are SH-orientated crosses (i.e. SH full siblings, and F_1 × F_1 SH♂SD♀). Gray squares and error bars are the means and 95% confidence intervals of each cross type.

and this approach retained a greater number of replicate familial lines across all lifecycle stages. The lack of apparent hybrid vigor among reciprocal SD × SH crosses might reflect the unique mutational loads of these populations, or the degree of divergence between them. Similarly, the manifestation and severity of outbreeding depression varies among F_1 × F_1 interpopulation crosses of *T. californicus* (Ellison and Burton 2008; Willett 2008; Olsen et al. 2023) and tends to worsen with increasing spatial and genetic divergence (Edmands 1999). Thus, the relative severity and timing of inbreeding and outbreeding depression described here may differ in crosses between less divergent populations of *T. californicus*. Nonetheless, reduced metamorphic rate is a pervasive consequence of F_1 × F_1 interpopulation crosses in *T. californicus*

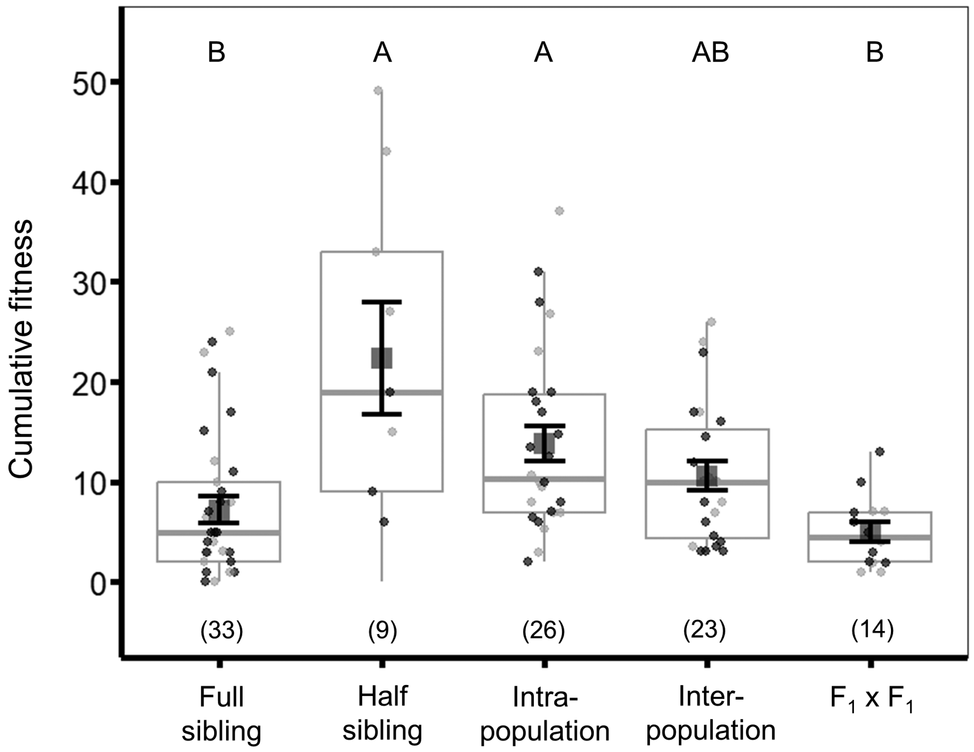

Fig. 4. Cumulative fitness effects of inbreeding and outbreeding in *Tigriopus californicus*. Cross types include full sibling, paternal half-sibling, unrelated intrapopulation, reciprocal interpopulation, and reciprocal F_1 × F_1 interpopulation crosses (SD♂ × SH♀ and SH♂ × SD♀). The letters A and B refer to the signifcance of between group comparisons. Black data points are SD-orientated crosses (i.e. within SD, SD♂ × SH♀, and F_1 × F_1 SD♂SH♀); gray data points are SH-orientated crosses (i.e. within SH, SH♂ × SD♀, and F_1 × F_1 SH♂SD♀). Boxes represent interquartile ranges, horizontal lines are medians, squares are means, and error bars are standard errors. Sample sizes in parentheses indicate the number of replicate familial lines evaluated in each cross type (Full sibling, Half sibling, Intrapopulation, and Interpopulation) and number of F_1 × F_1 combinations.

Table 1. Summary of competitive mating trials between *Tigriopus californicus* adult males produced from full sibling (inbred), intrapopulation, or F_1 × F_1 interpopulation (F_2) crosses and control males from culture.

Trial type	Trials	Clasping male
Inbred vs. control	20	10 inbred/10 control
Intrapopulation vs. control	10	5 intrapopulation/ 5 control
F_2 interpopulation vs. control	21	11 F_2 interpopulation/ 10 control

Inbred, intrapopulation, and F_2 interpopulation experimental males were equally as likely to clasp virgin females as control males.

and our results suggest inbreeding depression manifests in a similar form.

Our findings contrast the stage-specific consequences of inbreeding and outbreeding depression in exceptionally divergent allopatric populations. Han and Barreto (2021) found that divergence between SD and SH estimated at mtDNA protein-coding genes (11.1% to 35.2%) and nuclear genes (2.8% to 22.8%) was especially high and involved a substantial number of genetic elements. At this degree of divergence, incompatibilities between the mitochondrial and nuclear genomes have been shown to be disproportionately responsible for the inviability and phenotypic breakdown of *T. californicus* interpopulation hybrids (Burton and Barreto 2012; Healy and Burton 2020; Han and Barreto 2021). In comparison, the genetic basis of inbreeding depression is broadly tied to deleterious recessive alleles that are exposed in homozygous form (Charlesworth and Charlesworth 1999). As such, the severities of inbreeding and outbreeding depression detailed in our study likely reflect the relative magnitudes of genetic load present within populations and epistatic incompatibilities accrued between them. Given we detected weaker mean effects of inbreeding on metamorphic rate, greater variation in the effects of inbreeding among families, and rapid decay in the costs of inbreeding among paternal half-siblings, our results indicate deleterious recessives are less detrimental than epistatic incompatibilities at this scale of population divergence. Among populations with less spatial and genetic divergence, the contributions of epistatic incompatibilities (both nuclear–nuclear and mito–nuclear) to hybrid breakdown are reduced (Edmands 1999; Healy and Burton 2023), which may lessen differences in the severity and variability of inbreeding and outbreeding depression. Continued work focusing on mutational rates, purging, and the genomic causes of inbreeding depression in *T. californicus* could provide valuable insight into how demographic and evolutionary factors shape the relative abundances of these genetic elements.

The cumulative consequences of inbreeding and outbreeding depression are often tied to the environmental context (Edmands and Deimler 2004; Cheptou and Donohue 2011). Competition, predation, conspecific density, and environmental stress can exacerbate or dampen inbreeding and outbreeding depression (Plough 2012; Willett 2012; Pereira et al. 2014; Yun and Agrawal 2014). Mate choice in particular is greatly dependent upon the environmental context, as well as the distribution and characteristics of available mates (Olsen et al. 2021). Consequently, our findings under benign laboratory conditions may not exactly reflect the effects of inbreeding and outbreeding in nature. Even so, we detected no differences in the ability of inbred and outbred males to acquire female mates relative to control males in competitive trials. This result does not support the notion that inbreeding depression may be delayed to later stages of the lifecycle and expressed through reduced reproductive effort in *T. californicus* as it is in some flowering plants with a history of self-fertilization (Husband and Schemske 1996). Differences in the rate of purging between biparental and self-fertilization or in the equilibrium state of stage-specific deleterious recessives in the populations tested might account for this disparity. Alternatively, delayed inbreeding depression may have been expressed among other late-stage traits that we did not evaluate. Other post-metamorphic effects not characterized in our study such as the longevity, sterility, or fecundity of inbred and outbred individuals may also contribute to lifetime inbreeding and outbreeding depression (Flanagan et al. 2021; Olsen et al. 2023). Yet, these factors are unlikely to be as strong of selective forces as mortality prior to reproductive age and the effects of inbreeding and outbreeding on the metamorphic rate described here.

Owing to the rapid remodeling of the body plan, metamorphosis has been identified as an inherently vulnerable stage across animals with complex lifecycles (Lowe et al. 2021). Genetic and physiological elements responsible for low fitness and assembled by inbreeding and outbreeding may only become expressed during this transitional process. Our results point to the metamorphic transition as a key mechanism of both inbreeding and outbreeding depression in *T. californicus*. Similar patterns linking inbreeding depression to metamorphic success have been detailed in diverse species of amphibians, bryozoans, echinoderms, ascidians, and bivalves (Launey and Hedgecock 2001; Hughes et al. 2009; Ficetola et al. 2011; Plough and Hedgecock 2011; Zhao et al. 2016; Phillippi and Yund 2017). Evaluations of the relative timing of inbreeding and outbreeding depression in these species and others would shed light on the susceptibility of metamorphosis to inbreeding and outbreeding more broadly. Given our current findings and those across disparate taxa, stage-specific selection acting concurrently with the timing of metamorphosis may be a general factor influencing reproductive optima in species with biphasic larval/post-metamorphic life histories.

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

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Author contributions

Kevin Olsen (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft), Luis Escareno Medina (Data curation, Investigation, Writing – review & editing), Felipe Barreto (Conceptualization, Project administration, Supervision, Writing – review & editing), Suzanne Edmands (Conceptualization, Project administration, Supervision, Writing – review & editing), and Ronald Burton (Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing)

Data availability

The data supporting this article will be submitted to Dryad for public access upon publication.

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