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Citation for published version:

Richardson, J, Heinen-Kay, JL & Zuk, M 2021, 'Sex-specific associations between life-history traits and a novel reproductive polymorphism in the pacific field cricket', *Journal of Evolutionary Biology*, vol. 34, no. 3, pp. 549-557. <https://doi.org/10.1111/jeb.13758>

Digital Object Identifier (DOI):

[10.1111/jeb.13758](https://doi.org/10.1111/jeb.13758)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Evolutionary Biology

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Sex-specific associations between life-history traits and a novel reproductive polymorphism in the Pacific field cricket

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Funding information

Natural Environment Research Council, Grant/Award Number: NE/L002558/1; University of Minnesota

Abstract

Associations between heritable polymorphisms and life-history traits, such as development time or reproductive investment, may play an underappreciated role in maintaining polymorphic systems. This is because selection acting on a particular morph could be bolstered or disrupted by correlated changes in life history or vice versa. In a Hawaiian population of the Pacific field cricket (*Teleogryllus oceanicus*), a novel mutation (*flatwing*) on the X-chromosome is responsible for a heritable polymorphism in male wing structure. We used laboratory cricket colonies fixed for male wing morph to investigate whether males and females bearing the *flatwing* or *normal-wing* (wild-type) allele differed in their life-history traits. We found that *flatwing* males developed faster and had heavier testes than *normal-wings*, whereas *flatwing* homozygous females developed slower and had lighter reproductive tissues than *normal-wing* homozygous females. Our results advance our understanding of the evolution of polymorphisms by demonstrating that the genetic change responsible for a reproductive polymorphism can also have consequences for fundamental life-history traits in both males and females.

KEYWORDS

development time, life history, reproductive investment, reproductive polymorphism, *Teleogryllus oceanicus*

1 | INTRODUCTION

The coexistence of discrete morphs within one sex has been documented in a wide variety of animal species. Examples include differences in colour (e.g. Lank et al., 1995; Plaistow & Tsubaki, 2000; Svensson et al., 2005), size (e.g. Ryan et al., 1992; Shuster and Wade, 1991), weaponry (e.g. Painting et al., 2015; Radwan, 1993) and other morphological structures (e.g. Crespi, 1988; Zuk et al., 2006). In many cases, such distinct polymorphisms are also associated with alternative reproductive tactics, with different morphs using different strategies to secure access to mates (Brockmann, 2001; Gross, 1996;

Oliveira et al., 2008). For example, in Acarid mites, aggressive fighter males that use their sharply terminated legs to mortally stab other males coexist with unarmoured, benign scrambler males (Radwan, 2009). Similarly, throat patch colour is associated with male mating strategy in the side-blotched lizard (*Uta stansburiana*) with orange-throated males defending large territories, blue-throated males guarding single females and yellow-throated males acting as nonterritorial 'sneakers' (Sinervo & Lively, 1996). Understanding how such polymorphisms arise and are maintained is an important focus of evolutionary biology as polymorphic systems provide critical insights into the processes underlying genetic and phenotypic diversity within species (Galeotti et al., 2003; Heinen-Kay et al., 2020;

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McKinnon & Pierotti, 2010) and even the generation of new species (Hugall and Stuart Fox, 2012).

Maintenance of multiple discrete morphs is typically explained by negative frequency-dependent selection, with rare morphs having an advantage due to biotic processes such as predation (Davison, 2002), parasitism (Losey et al., 1997) or sexual selection (Nielsen & Watt, 2000). However, selection acting on a particular morph (or its associated mating strategy) will often have consequences for other traits. This can occur because heritable polymorphisms are correlated with other traits, such as life-history traits, which are unrelated to morph per se but are also under selection. Examples of such traits include development time, growth rate, fecundity or disease resistance, all of which have been found to vary between morphs in different species (Abbott & Svensson, 2005; Bots et al., 2009; Svensson et al., 2001; Wilson et al., 2001). These correlations are important because they may hinder or facilitate the processes maintaining polymorphisms. Selection acting on a particular morph will lead to correlated changes in life-history traits which could, in turn, strengthen or weaken the selective advantage of that morph—especially when populations are exposed to environmental or social conditions that select for particular life-history strategies. Furthermore, although heritable polymorphisms are often expressed in only one sex, the alleles that control morph development may be present in the other sex (Plesnar-Bielak et al., 2014; Robinson et al., 2006). If this is the case, selection on correlated life-history traits in the nonpolymorphic sex could further contribute to hindering or facilitating maintenance of the polymorphism. Thus, investigating associations between heritable polymorphisms and life-history traits in both the polymorphic and nonpolymorphic sex will provide valuable insights into the evolution and maintenance of polymorphisms.

Pacific field crickets (*Teleogryllus oceanicus*) provide a unique opportunity to investigate associations between polymorphism and life-history traits because males in some populations of this species exhibit a heritable, discontinuous wing polymorphism. Like many crickets, *T. oceanicus* males call to attract females (Otte, 1994; Zuk & Simmons, 1997). In Hawaii, *T. oceanicus* co-occurs with a parasitoid fly, *Ormia ochracea*, which exploits male songs to locate a host for their larvae, the development of which ultimately kills the cricket (Cade, 1975; Zuk et al., 1993; Adamo et al., 1995; Wagner, 1996). Between 2001 and 2003, a mutation that erases the stridulatory apparatus from male forewings arose and rapidly spread to ~90% of the males on Kauai (Zuk et al., 2006), and has remained stable at approximately this proportion ever since (Zuk et al., 2006, 2018). Because these males lack sound-producing structures, they are rendered obligately silent, and their morph is referred to as 'flatwing'. Wild-type morphs that possess stridulatory wing structures and can sing are called 'normal-wings'. Male wing morph is subject to strong trade-offs between natural and sexual selection. Flatwing males avoid detection by the parasitoid and likely experience greater survival as a result (Zuk et al., 2006). However, flatwing males face difficulty attracting mates because they cannot produce the calling or courtship songs that females find attractive (Bailey & Zuk, 2012; Tinghitella &

Zuk, 2009). Indeed, flatwing males can likely only achieve matings by adopting an alternative mating tactic in which they intercept females that are attracted to the caller's song (Bailey et al., 2010; Olzer & Zuk, 2018; Zuk et al., 2006).

Flatwing males experience other effects of the wing polymorphism, including differences in gene expression (Pascoal et al., 2016), cuticular hydrocarbons (Simmons et al., 2014) and reproductive physiology—flatwing males have lighter testes (Bailey et al., 2010; Rayner et al., 2019), but sire more offspring per mating than normal-wings (Heinen-Kay, Urquhart et al., 2019). Flatwing morphology is caused by a mutation on the X-chromosome that segregates at a single locus (Tinghitella, 2008). Given that sex determination is XX/XO in crickets, males possess a single copy of the allele, determining their wing morph, and females carry two copies. Although females do not express any wing traits associated with the mutation, being homozygous for the flatwing and normal-wing alleles is associated with a host of effects in females. These include differences in gene expression (Pascoal et al., 2018; Rayner et al., 2019), reproductive investment and mating behaviour—flatwing homozygous females invest less in reproductive tissues (Heinen-Kay et al., 2019) and show a lower propensity to mount males (Heinen-Kay et al., 2020). This combination of advantageous and detrimental effects of the flatwing mutation in both males and females appears to contribute to the maintenance of the wing polymorphism. Our study aimed to investigate whether similar associations occur for fundamental life-history traits such as development time and body size. If we find that carrying the flatwing allele also elicits beneficial or detrimental effects on life history, this would provide additional evidence for the broad, pleiotropic effects of the novel mutation in this system and advance our understanding of the processes maintaining this polymorphism in natural populations.

We investigated associations between wing polymorphism and life history in flatwing and normal-wing males and females homozygous for the flatwing or normal-wing allele. To accomplish this, we used previously established flatwing and normal-wing colonies that produce only one male wing morph—demonstrating that the females in these colonies are all homozygous for the allele associated with each morph (Heinen-Kay, Strub, et al., 2019). This approach allowed us to investigate associations between wing polymorphism and life-history traits in males and females without requiring molecular assays or controlled breeding. Here we investigated whether wing polymorphism was associated with differences in development time. Differences in development time occur in a number of polymorphic insects (Abbott & Svensson, 2005; Ahnesjö & Forsman, 2003; Cook & Jacobs, 1983), and can have important consequences for polymorphisms. For example, in the damselfly *Ischnura elegans*, colour morph is correlated with development time in both trimorphic females and monomorphic males, meaning that selection for early emergence in either sex could drive changes in morph frequency (Abbott & Svensson, 2005). Development time is an important component of fitness in many animals (e.g. Holzapfel & Bradshaw, 2002; Moynihan & Shuker, 2011; Plaistow & Siva-Jothy, 1999; Semlitsch et al., 1988), in particular because developing faster is often beneficial for

reproductive competition (Morbey and Ydenberg, 2001). In addition, faster development time may be under selection because it leads to a shorter generation time and therefore a faster rate of reproduction in growing populations. On the other hand, a slower development time may be beneficial if developing quickly necessitates trade-offs with other life-history functions such as growth, survival and reproduction (Roff, 2000; Stearns, 1992). Thus, to fully investigate the consequences of putative differences in development time between *flatwing* and *normal-wings* we also measured differences in juvenile and adult body size, survival to eclosion, and investment to reproductive tissues in *flatwing* and *normal-wing* males and females.

2 | METHODS

2.1 | Colony construction and cricket maintenance

Our study used *T. oceanicus* from separate outbred *flatwing* and *normal-wing* colonies. These single allele colonies are descended from a Kauai colony that was founded in 2003, after discovery of *flatwing*, and has been supplemented with eggs from the wild at least annually. For full details on the construction of the *flatwing* and *normal-wing* colonies, see Heinen-Kay, Strub, et al. (2019). Briefly, individual females from the Kauai laboratory stock were mated with a single normal-wing or flatwing male and, for the resulting F1 offspring, male phenotype and parental male phenotype were used to determine parental female genotype. Homozygous F1 female offspring were then mated with a single male with the corresponding wing morph from the Kauai colony to produce F2 lines that were combined to generate pure-breeding colonies. Males from these colonies always breed true for wing morph—indicating that females from each colony are homozygous for the respective allele. The *flatwing* and *normal-wing* colonies are reared in the same Caron Insect Growth Chamber which maintains a 26°C, 75% humidity environment and a photo-reversed 12:12 light-dark cycle. Crickets in these colonies are housed in multiple 15-L plastic containers which are kept at a consistent density and provided with ad lib access to commercial rabbit food, moist cotton for water and oviposition, and egg carton for shelter.

2.2 | Experimental procedures

We isolated 41 male and 40 female juveniles from the *flatwing* colony and 48 male and 48 female juveniles from the *normal-wing* colony as soon as sex differences were evident. This is the earliest stage at which individuals can be kept in isolation without experiencing high mortality. To minimize variation in age and developmental stage at the start of the experiment, we only selected juvenile females that had an ovipositor smaller than 2 mm and juvenile males that had only developed the first pair of wing buds. We moved each juvenile to individual 118-mL cups containing food, water and egg carton for shelter, and housed them in an incubator set to 27°C, 75% relative

humidity, and a photo-reversed 12:12 light:dark cycle. This incubator also housed a colony containing naturally singing males so that all crickets used in our study were exposed to similar levels of conspecific song during development. We did this to control for the effects of variation in acoustic experience on life-history traits such as development time and reproductive physiology (Bailey et al., 2010; Kasumovic et al., 2011).

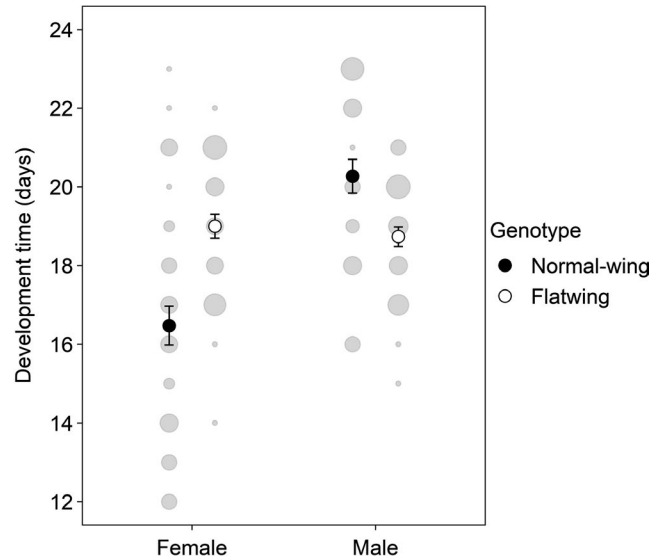
When isolating individuals as juveniles, we recorded their body weight to the nearest 0.01 mg using a Sartorius electric balance and their pronotum width to the nearest 0.01 mm using digital callipers as measures of juvenile body size. To measure development time, we checked crickets daily for adult eclosion and recorded the number of days from separation to eclosion. We measured survival to eclosion by recording all incidences of death prior to adult eclosion ($n = 27$). On the day of adult eclosion, we again measured the body weight and pronotum width of each individual as measures of adult body size. After eclosion, we left crickets for a further 6–8 days to reach sexually maturity.

We next mated each of our focal individuals to a haphazardly chosen, opposite sex *normal-wing* individual from the general Kauai laboratory colony. We did this to ensure that all individuals had experienced mating prior to dissection of their reproductive tissues. Matings took place during the crickets' normal active period (09:00–21:00) in an anechoic chamber under red light. We initiated mating by placing each focal individual and their assigned mate in a 118-ml cup. Because females are unlikely to mate in the absence of courtship song (Kota et al., 2020; Tinghitella & Zuk, 2009), we exposed all pairs to playback of courtship song. The playback consisted of a continuous loop of a courtship song recorded from the Kauai population broadcast at 70–75 dB SPL (sound pressure level) measured at 10 cm from the source. To prevent interference between the playback and a male's own courtship song, *normal-wing* males were surgically silenced prior to mating by removing the sound-producing structure (scraper) from the forewing with microsurgical scissors. Removal of the scraper has no discernible effect on male behaviour (Bailey et al. 2008; Balenger et al., 2018). Nevertheless, to control for any effects of handling or wing cutting, we also removed the same section of the forewing from *flatwing* males. We left pairs for 10 min after which we checked for successful mating as indicated by the presence of a spermatophore attached to the female. If pairs did not mate after 10 min, we removed the mate and left the focal male or female alone in the cup for 10 min in a quiet, dark space before providing a new mate from the general Kauai colony. We repeated this process up to a maximum of three times until all pairs had successfully mated. We confirm that all individuals ($n = 150$) successfully mated within three attempts. After successful mating, focal individuals were again isolated in individual 118-mL cups containing food, water and egg carton and returned to the incubator for 48 hr. At this point, individuals were killed by freezing and then dissected the following day. We measured wet weight of the whole, intact body before carefully removing reproductive tissues (i.e. testes or eggs and ovarioles) and recording their wet weight to the nearest

TABLE 1 Effects of sex, genotype (flatwing or normal-wing) and their interaction on life-history traits in *Teleogryllus oceanicus*

	Development time (days)			Adult pronotum width (mm)			Adult body mass (mg)			Survival to eclosion			Reproductive tissue mass (mg)		
	Est ± SE	t	p	Est ± SE	t	p	Est ± SE	t	p	Est ± SE	z	p	Est ± SE	t	p
Sex	-0.37 ± 0.46	-0.80	.42	0.091 ± 0.056	1.62	.10	29.9 ± 13.6	2.20	.028	-1.12 ± 1.17	-0.95	.33	-87.6 ± 4.81	-18.2	<.001
Genotype	2.17 ± 0.46	4.66	<.001	0.22 ± 0.055	4.15	<.001	57.2 ± 13.5	4.23	<.001	2.05 ± 1.08	1.89	.058	-13.7 ± 4.68	-2.92	.0040
Sex × genotype	-3.99 ± 0.67	-5.96	<.001	0.14 ± 0.081	1.81	.071	25.9 ± 19.5	1.32	.18	-0.30 ± 1.27	-0.23	.81	15.2 ± 6.64	3.21	.023
Juvenile body mass (mg)	-0.022 ± 0.0033	-6.66	<.001	-	-	-	0.36 ± 0.10	3.76	<.001	-	-	-	-	-	-
Juvenile pronotum width (mm)	-	-	-	0.72 ± 0.10	7.04	<.001	-	-	-	-	-	-	-	-	-
Somatic mass (mg)	-	-	-	-	-	-	-	-	-	-	-	-	0.084 ± 0.026	3.21	.0017

Note: We provide parameter estimates (Est), standard errors (SE), test statistics (*t/z*) and *p*-values for each trait. Female and normal-wing were used as the reference levels for sex and genotype, respectively. Significant *p*-values are indicated in bold type

**FIGURE 1** Differences in the development time of female and male *Teleogryllus oceanicus* from fixed genotype colonies. Filled symbols represent means ± SE for crickets carrying the *normal-wing* allele, whereas open symbols represent means ± SE for crickets carrying the *flatwing* allele. Grey circles represent data on individual crickets with the size of the circle representing the frequency of observations

0.01 mg. We later subtracted our measure of reproductive tissue mass from our measure of whole-body mass to estimate the mass of nonreproductive tissue (i.e. somatic mass).

2.3 | Statistical analyses

We investigated whether the *flatwing* or *normal-wing* genotype was associated with differences in development time, survival to eclosion, adult body size and reproductive tissue mass. We used general linear models to test for differences in development time, adult body size (i.e. adult pronotum width and adult body mass) and reproductive tissue mass. To test for differences in survival to eclosion (survived or died), we used a binomial generalized linear model. All models included sex, genotype (*flatwing* or *normal-wing*) and the sex-by-genotype interaction as fixed effects.

Furthermore, the model of development time included juvenile body mass as a covariate to control for differences in juvenile body size on development time. However, the results were qualitatively similar if we instead included juvenile pronotum width as a covariate. The model of adult body mass included juvenile body mass as a covariate, whereas the model of adult pronotum width included juvenile pronotum width as a covariate. Finally, the model of reproductive tissue mass included somatic mass (i.e. whole-body mass minus reproductive tissue mass) as a covariate to control for variation in reproductive investment due to body size. All analyses were performed in R statistical software version 3.6.0 (R Core Team, 2019).

3 | RESULTS

3.1 | Development time

Development time was significantly associated with the interaction between sex and genotype (*normal-wing* or *flatwing*) (Table 1). This interaction effect reflected that the *flatwing* allele was associated with faster development in males but slower development in females (Figure 1). *Flatwing* males reached adulthood, on average, 7.6% faster than *normal-wing* males, whereas *normal-wing* homozygous females reached adulthood, on average, 15.3% faster than *flatwing* homozygous females. Overall, there was a significant association between genotype and development time, with the *normal-wing* genotype developing faster (Table 1). However, there was no main effect of sex on development time (Table 1). Finally, a heavier juvenile body mass was associated with faster development time (Table 1).

3.2 | Body mass and pronotum width

At the juvenile stage, there was no difference between the body mass of males and females ($F_{1,173} = 0.097, p = .75$), but males were larger than females in terms of pronotum width ($F_{1,172} = 9.67, p = .0022$). Additionally, there was no difference between *normal-wings* and *flatwings* in terms of juvenile body mass ($F_{1,173} = 0.91, p = .34$) or juvenile pronotum width ($F_{1,172} = 2.79, p = .096$). Furthermore, there was no interaction between sex and genotype for either juvenile body mass ($F_{1,173} = 0.025, p = .34$) or juvenile pronotum width ($F_{1,172} = 1.71, p = .19$).

When controlling for juvenile pronotum width, the *flatwing* genotype was associated with a significantly larger adult pronotum width than the *normal-wing* genotype in both sexes (Table 1; Figure 2a). The pronotum width of *flatwings* at eclosion was, on average, 6.0% larger than the pronotum width of *normal-wings*. In addition, adult pronotum width increased with increasing juvenile pronotum width

(Table 1). However, there was no effect of sex, or the interaction between sex and genotype, on adult pronotum width (Table 1). Similarly, the *flatwing* genotype was associated with a significantly heavier body mass as an adult compared to the *normal-wing* genotype (Table 1; Figure 2b). *Flatwings* were, on average, 14.2% heavier than *normal-wings* at eclosion. Furthermore, males were, on average, significantly heavier than females, but there was no evidence for an interaction between sex and genotype on adult body mass (Table 1). In addition, increasing juvenile body mass was associated with a significant increase in adult body mass (Table 1).

3.3 | Survival to eclosion

There was no evidence that survival to eclosion was significantly associated with sex, genotype or the interaction between sex and genotype (Table 1). *Normal-wings* were somewhat less likely to survive to eclosion than *flatwings*, but this trend was not statistically significant (Table 1).

3.4 | Reproductive tissue mass

Reproductive tissue mass was significantly associated with the interaction between sex and genotype (Table 1; Figure 3). This interaction reflected that *flatwing* homozygous females had significantly lighter reproductive tissues than *normal-wing* homozygous females (Figure 3a). However, for males, the opposite pattern was observed, with *flatwing* males having significantly heavier reproductive tissues than *normal-wing* males (Figure 3b). Reproductive tissues were, on average, 8.9% heavier in *normal-wing* homozygous females than *flatwing* homozygous females, whereas testes were, on average, 22.6% heavier in *flatwing* males than *normal-wing* males. Finally, as expected, reproductive tissue mass significantly increased with increasing somatic mass (Table 1).

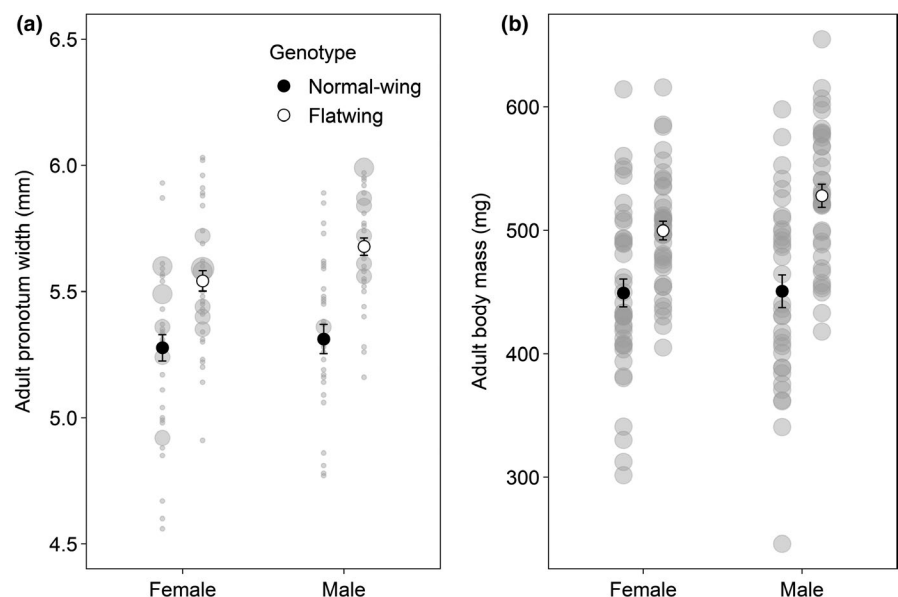


FIGURE 2 Differences in adult pronotum width (a) and adult body mass (b) of female and male *Teleogryllus oceanicus* from fixed genotype colonies. Filled symbols represent means \pm SE for crickets carrying the *normal-wing* allele, whereas open symbols represent means \pm SE for crickets carrying the *flatwing* allele. Grey circles represent data on individual crickets with the size of the circle representing the frequency of observations

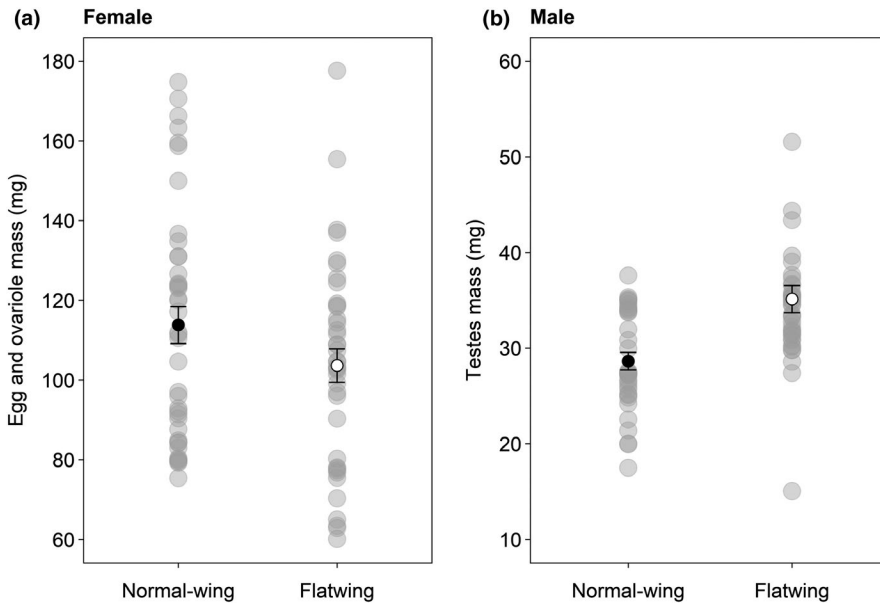


FIGURE 3 Differences in the mass of reproductive tissues of female (a) and male (b) *Teleogryllus oceanicus* from fixed genotype colonies. Filled symbols represent means \pm SE for crickets carrying the *normal-wing* allele, whereas open symbols represent means \pm SE for crickets carrying the *flatwing* allele. Grey circles represent data on individual crickets with the size of the circle representing the frequency of observations

4 | DISCUSSION

Our study investigated associations between a heritable reproductive polymorphism and life-history traits in the Pacific field cricket, *Teleogryllus oceanicus*. We found that the allele responsible for male wing morph was associated with differences in development time, adult body size and reproductive tissue investment, but that, in some traits, these differences manifested in opposite directions in males and females. *Flatwing* males developed faster and had heavier testes than *normal-wings*, whereas *flatwing* homozygous females developed slower and had lighter eggs and ovarioles than *normal-wing* homozygous females. Finally, *flatwing* was associated with a larger adult body size in both sexes. Such associations between the male wing polymorphism and life-history traits may have important implications for evolutionary dynamics and the maintenance of polymorphisms in natural populations, because selection acting to hinder or facilitate the spread of *flatwing* will lead to correlated changes in life history (or vice versa) and thus influence the fitness of each morph. These results advance our understanding of the evolution of polymorphisms by demonstrating that the genetic change responsible for a reproductive polymorphism can also have consequences for life-history traits.

The *flatwing* mutation was associated with differences in development time. *Flatwing* males developed quicker than *normal-wing* males, whereas *normal-wing* homozygous females developed quicker than *flatwing* homozygous females. It is important to note that our study examined differences in development time from the early juvenile stage until adult eclosion. This is because it is not possible to isolate individuals from hatching without high mortality. Thus, we cannot exclude the possibility that differences in development time also occur during earlier life stage such as during embryonic development or between hatching and early instars. Development time is important for fitness in many animals. For example, faster development is often beneficial to male mating success because emerging

earlier allows males to monopolize access to females (Morbey & Ydenberg, 2001). However, given that *T. oceanicus* have overlapping generations and breed continuously (Simmons & Zuk, 1994), developing more quickly is unlikely to provide a mating advantage. Another potential consequence of differences in development time between *flatwings* and *normal-wings* is that faster development will shorten generation time in one morph relative to the other. This could have important consequences for the fitness of each morph because *T. oceanicus* breed continuously, meaning that the faster developing morph will produce more generations in a given period of time and, all else being equal, may ultimately achieve higher fitness.

Conversely, slower development can be beneficial if it allows individuals to grow larger or increase their investment to functions such as reproduction (Roff, 2000). However, we found no evidence for such trade-offs as the genotype that developed more quickly also had heavier reproductive tissues in both sexes. Similarly, the *flatwing* genotype was associated with larger adult size, even though *flatwing* males developed faster than *normal-wing* males. Potentially, faster development may be associated with costs that our study did not measure. For example, faster development impaired starvation resistance in speckled wood butterflies, *Pararge aegeria* (Gotthard et al., 1994). Similarly, in the neriid fly, *Telostylinus angusticollis*, high-condition males develop faster but experience an increased rate of reproductive senescence (Hooper et al. 2017). Although we cannot determine the ultimate fitness consequences of differences in development time in *T. oceanicus*, our study demonstrates a novel consequence of carrying the *flatwing* allele on a fundamental life-history trait.

Flatwing was also associated with differences in reproductive tissues in both males and females. The eggs and ovarioles of *flatwing* homozygous females weighed considerably less than those of *normal-wing* females. In contrast, the testes of *flatwing* males were significantly heavier than those of *normal-wing* males. Our findings corroborate previous work showing that being homozygous for the

flatwing allele involves a direct cost to female reproductive capacity (Heinen-Kay, Strub, et al., 2019). However, prior work also found that *flatwing* and *normal-wing* females produce a similar number of offspring (Heinen-Kay, Strub, et al., 2019), suggesting that increased reproductive capacity may trade off with other aspects of reproduction such as egg size or quality. Furthermore, *flatwing* homozygous females are less likely to mount males than *normal-wing* homozygous females (Heinen-Kay, Urquhart, et al., 2019). Thus, although relative reproductive tissue mass provides a useful snap-shot estimate of reproductive investment (Heinen-Kay, Strub, et al., 2019; Zuk et al., 2004), such differences may not reflect actual reproductive outcomes given that females can also differ in their fecundity or behaviour.

In contrast, males benefitted from the *flatwing* mutation through increased reproductive investment. Given that *flatwings* cannot sing to attract females (Bailey & Zuk, 2012; Tinghitella & Zuk, 2009), and are discriminated against during mating interactions by females (Tinghitella & Zuk, 2009), increased investment to reproductive tissues may allow *flatwing* males to make the most of their limited mating opportunities. In support of this, prior work found that *flatwing* males sire more offspring per mating event than *normal-wings* (Heinen-Kay, Strub, et al., 2019). The increased investment to reproductive tissues by *flatwing* males may reflect a shift in resource allocation away from other functions such as signalling or immunity. However, although they lack sound-producing structures, *flatwing* males still engage in energy-intensive stridulation (Schneider et al., 2018) and there is no difference in calling effort between *flatwing* and *normal-wing* males (Rayner et al., 2020). In addition, *flatwing* males have stronger immune responses than *normal-wing* males (Bailey et al., 2011). Thus, it is unlikely that heavier testes represent an adaptive reallocation of resources by *flatwings*. Furthermore, we note that our results are in contrast to prior work which found significantly *lighter* testes in *flatwing* males compared to *normal-wings* (Bailey et al., 2010; Rayner et al., 2019). The reason for these contradictory results is unclear. Potentially, these contradictory patterns may reflect differences in the source populations used to establish laboratory colonies. For example, differences in population density, genetic composition, environmental conditions or selection pressures could influence the association between genotypes and life-history traits. More work is required to understand these contradictory findings, but they do highlight that the pleiotropic effects of the *flatwing* allele on reproductive investment have the potential to be remarkably plastic.

We found evidence for sex-by-genotype interactions in the association between the *flatwing* allele and life-history traits. *Flatwing* was associated with faster development and heavier reproductive tissues in males, but slower development and lighter reproductive tissues in females. An allele can be beneficial in both sexes, thereby favouring its spread, or it can be beneficial in one sex and costly in the other, generating intralocus sexual conflict (Bonduriansky & Chenoweth, 2009). Thus, the opposing effects of the *flatwing* allele on male and female life-history traits may function as a brake on the spread of *flatwing* and help maintain the polymorphism. Somewhat

counterintuitively, these factors that maintain the polymorphism may also ensure the persistence of *flatwing* males in wild populations. This is because *flatwing* males must rely on the remaining callers to use their satellite mating tactic. As calling song is the primary mechanism for mate localization, without *normal-wing* males the sexes would be largely unable to find each other. Thus, population viability likely depends on *flatwing* not becoming fixed. If both males and females benefitted from carrying the *flatwing* allele, it might spread to the point of wiping out *normal-wing* males and dooming the population, whereas antagonistic effects would allow the polymorphism to be maintained. Although for some traits (e.g. development time) we cannot determine whether the particular associations we find are beneficial or costly, our results add to previous work showing that the consequences of carrying the *flatwing* allele for females are integral to the maintenance of the reproductive polymorphism in males (Heinen-Kay et al., 2020; Heinen-Kay, Strub, et al., 2019).

Our results bolster prior work showing that an important consequence of the evolution of a novel polymorphism in *T. oceanicus* is the correlations between the *flatwing* mutation and suites of other traits. For example, *flatwing* is linked with differences in cuticular hydrocarbons (Simmons et al., 2014), gene expression (Pascoal et al., 2016; Rayner et al., 2019), reproductive physiology (Bailey et al., 2010; Heinen-Kay, Strub, et al., 2019), male reproductive success (Heinen-Kay, Urquhart, et al., 2019) and female mating behaviour (Heinen-Kay et al., 2020). The unique contribution of our results is to demonstrate that *flatwing* is also associated with differences in development time and body size. These associations are important because selection favouring or hindering the spread of *flatwing* will lead to correlated changes in life histories. Such differences in life-history traits could then affect the fitness of each morph and ultimately strengthen or disrupt the processes maintaining the polymorphism in wild populations. Since population viability seemingly depends on males remaining polymorphic, understanding the broader consequences associated with the *flatwing* allele could be particularly important in this system. Our study advances our understanding of polymorphisms by demonstrating that the evolution of a novel reproductive polymorphism that silences males also has consequences for male and female life-history traits. Potentially, such associations may play an underappreciated role in the maintenance of polymorphisms.

ACKNOWLEDGMENTS

We are incredibly grateful to Rachel Nichols for her assistance with setting up the experiment. We thank Kristin Robinson, Taren Stanley, Liz Wild, Robin Mullard, Sara de Sobrino and Kristine Jecha for assistance with cricket colony maintenance. We thank three anonymous reviewers for their helpful comments on the manuscript. We are also grateful to funds from the University of Minnesota and the Overseas Research Visit and Conference Fund (ORVCF) and Professional Internship Programme (PIP) made available by the E3/E4 doctoral training partnership at The University of Edinburgh. JR is supported by a Natural Environment Research Council (NERC) doctoral training partnership grant (NE/L002558/1).

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13758>.

DATA AVAILABILITY STATEMENT

Analyses reported in this article can be reproduced using the data and code deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.h70rxwdhh>).

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How to cite this article: Richardson J, Heinen-Kay JL, Zuk M. Sex-specific associations between life-history traits and a novel reproductive polymorphism in the Pacific field cricket. *J Evol Biol.* 2021;34:549–557. <https://doi.org/10.1111/jeb.13758>