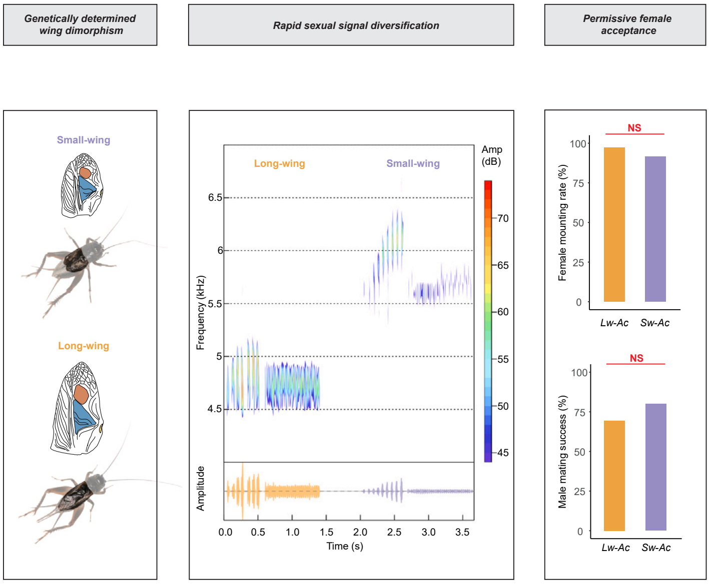
Report

Current Biology

Rapid sexual signal diversification is facilitated by permissive females

Graphical abstract



Highlights

- Sexual signal evolution requires that novel traits escape loss due to selection
- In Hawaiian crickets, small wings evolved several years ago, diversifying male song
- Females mate with singing small-wing males as readily as with long-wing males
- Permissive female mating can facilitate rapid sexual trait diversification

Authors

Renjie Zhang, Jack G. Rayner, Nathan W. Bailey

Correspondence

rz40@st-andrews.ac.uk (R.Z.),
nwb3@st-andrews.ac.uk (N.W.B.)

In brief

Zhang et al. test how new animal signal variants evolve. In Hawaiian crickets, males evolved higher-pitch courtship songs under selection from eavesdropping enemies. However, males singing songs at these abnormal frequencies suffer no mating disadvantage. Permissive females may buffer against the loss of new signal variants.

Zhang et al., 2024, Current Biology *34*, 403–409
January 22, 2024
https://doi.org/10.1016/j.cub.2023.11.063

Current Biology

CellPress
OPEN ACCESS

Report

Rapid sexual signal diversification is facilitated by permissive females

Renjie Zhang,[1,3,4,*] Jack G. Rayner,[2] and Nathan W. Bailey[1,*]
[1]School of Biology, University of St Andrews, St Andrews, Fife KY16 9TH, UK
[2]Department of Biology, University of Maryland, College Park, MD 20741, USA
[3]X (formerly Twitter): @Renjie__Zhang
[4]Lead contact
*Correspondence: rz40@st-andrews.ac.uk (R.Z.), nwb3@st-andrews.ac.uk (N.W.B.)
https://doi.org/10.1016/j.cub.2023.11.063

SUMMARY

The initial process by which novel sexual signals evolve remains unclear, because rare new variants are susceptible to loss by drift or counterselection imposed by prevailing female preferences.[1–4] We describe the diversification of an acoustic male courtship signal in Hawaiian populations of the field cricket *Teleogryllus oceanicus*, which was brought about by the evolution of a brachypterous wing morph ("small-wing") only 6 years ago.[5] Small-wing has a genetic basis and causes silence or reduced-amplitude signaling by miniaturizing male forewings, conferring protection against an eavesdropping parasitoid, *Ormia ochracea*.[5] We found that wing reduction notably increases the fundamental frequency of courtship song from an average of 5.1 kHz to 6.4 kHz. It also de-canalizes male song, broadening the range of peak signal frequencies well outside normal song character space. As courtship song prompts female mounting and is sexually selected,[6–9] we evaluated two scenarios to test the fate of these new signal values. Females might show reduced acceptance of small-wing males, imposing counterselection via prevailing preferences. Alternatively, females might accept small-wing males as readily as long-wing males if their window of preference is sufficiently wide. Our results support the latter. Females preferred males who produced some signal over none, but they mounted sound-producing small-wing males as often as sound-producing long-wing males. Indiscriminate mating can facilitate the persistence of rare, novel signal values. If female permissiveness is a general characteristic of the earliest stages of sexual signal evolution, then taxa with low female mate acceptance thresholds should be more prone to diversification via sexual selection.

RESULTS AND DISCUSSION

Adaptive sexual signal reduction in Hawaiian crickets

Sexual signal character changes are inherent to models of speciation via sexual selection,[10,11] but a major unanswered question is how novel signal variants become established at their inception.[1–4] Consider a population where signal and receiver traits are at a stable equilibrium. When a novel trait invades through *de novo* mutation or introgression and is thus very rare, virtually all individuals of the opposite sex would be expected to disfavor it. One largely overlooked idea is that a wide window of mate acceptance could allow the persistence of such novel sexual trait values.[12–14] Testing this requires assessing the very earliest stages of evolved sexual character change—an event that is vanishingly rare on contemporary timescales and thus infrequently observed in nature. We overcame these challenges by capitalizing on an example of rapid, recent, sexual trait character change in the field cricket *Teleogryllus oceanicus*.

Male crickets sing to attract females and induce mounting. The carrier, or dominant, frequency of male song shows consistent inter-species differences, and frequency shifts have occurred throughout the evolutionary history of crickets.[15–18] In a *T. oceanicus* population on the Big Island of Hawaii, we discovered a novel brachypterous male cricket phenotype—"small-wing" (Figure 1A). We had not observed the small-wing phenotype prior to 2017, despite over a decade of monitoring,[19] and brachyptery has not to our knowledge been reported in *T. oceanicus*. Crickets sing by rubbing specialized structures on their forewings together (Figure 1B), but small-wing males are either unable to produce any sound, or they produce a sound with dramatically different characteristics. Small-wing is genetically encoded, expressed by both sexes, and appears to protect males from an eavesdropping parasitoid fly, *Ormia ochracea*.[5] It differs from other male-silencing wing variants in Hawaiian *T. oceanicus*, the best studied of which is "flatwing," and thus represents a parallel anti-parasitoid defense.[5]

Morphological basis of recently evolved sexual signal change

We first confirmed that the forewings of small-wing individuals are smaller than those of long-wing individuals (Figures 1B and 1C). Distributions of the right forewing length from 653 individuals of both phenotypes and sexes were non-overlapping, and wing length of small-wing individuals was approximately two-thirds that of long-wing individuals (Welch's t tests: males: df = 329.08, t = 49.36, $p < 0.001$; females: df = 65.94, t = 29.35,

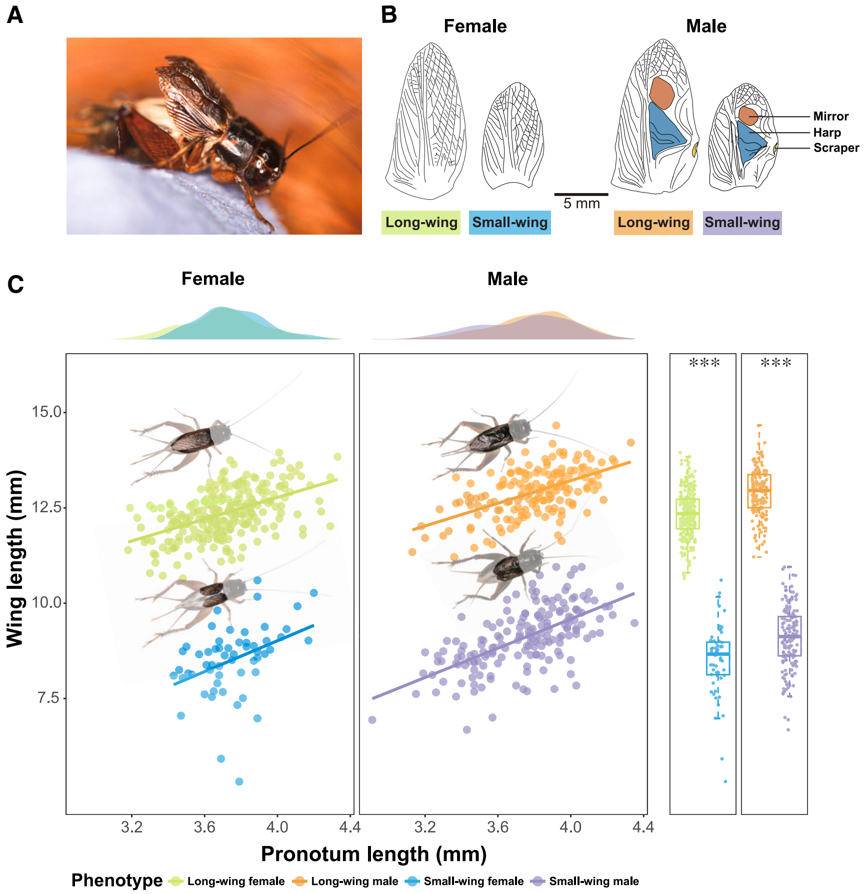


Figure 1. Morphological basis of altered male song in *T. oceanicus*

(A) A singing small-wing *T. oceanicus* male.

(B) Diagram of female (left) and male (right) forewings of each morph. Important resonating structures on male wings are highlighted (mirror, orange; harp, blue; scraper, yellow).

(C) Relationship between forewing and pronotum length within each sex and morph. Best fit lines are indicated for each sex and morph, respectively. Cricket forewings are highlighted for visual clarity using Adobe Photoshop 2023. Density plots above the panels show the distribution of pronotum length, and boxplots to the right show wing length comparisons within each sex, respectively. Asterisks indicate statistical significance; see main text for details.

$p < 0.001$) (Figure 1C; Table 1). Although reduced wing size is inherent to the definition of the phenotype,[5] the non-overlapping dimorphism within each sex is more discrete than that observed in some other wing-polymorphic insects. For example, brachyptery in the sister species *Teleogryllus derelictus* occurs with intermediate forms,[20] and intermediate wing lengths result from hybridization in the beetle *Galerucella grisescens*.[21]

We found no evidence for condition dependence of small-wing expression. Within each sex, the linear relationship between wing length and body size was similar for both morphs (pronotum length*morph interactions from linear models: males, $F_{1,339} = 2.75$, $p = 0.098$; females, $F_{1,306} = 1.47$, $p = 0.226$), and the distribution of pronotum lengths overlapped for both forms within each sex (Welch's t tests: males, df = 339.89, t = 1.78, $p = 0.076$; females, df = 94.58, $t = -1.58$, $p = 0.117$) (Figure 1C; Table 1). We would expect neither if adult wing polymorphism was condition-dependent.[22] Insect wing polymorphism is frequently invoked as an example of adaptive plasticity underlying ecological success in heterogeneous environments.[23] Brachyptery in grylline crickets principally affects the hindwings rather than the forewings, which enables continued generation of acoustic signals.[24] However, the forewing veins and cells that produce sound in male small-wing *T. oceanicus* are miniaturized versions of long-wing males', a difference conspicuous to the naked eye (Figure 1B). Small-wing thus resembles other species with constitutively short forewings, such as *Gryllus ovisopis*. To test the functional consequences of miniaturized forewings, we next analyzed acoustic recordings from males in courtship trials.

Small-wing morphology substantially changes male song

We focused on courtship song, rather than long-range calling song, due to the former's role in mate evaluation and acceptance during close-range reproductive interactions.[6–9] In mating trials, approximately one-eighth (n = 8) of small-wing males were completely silent despite still expressing wing movements associated with singing (Video S3). The remainder (87.30%, n = 55) produced audible courtship songs, 80% of which were below the lowest peak amplitude of long-wing males (n = 44, 8 of them were removed from further analysis; see STAR Methods for details). Song structure was similar for both morphs (Figure 2A), consisting of a short, higher-amplitude series of chirps followed by a long constant trill.[6] However, the morphs differed in both carrier frequency and peak amplitude. Small-wing males produced higher carrier frequencies (Wilcoxon rank-sum test: W = 1,661; $p < 0.001$) with a far broader range (Levene's test: $F_{1,83} = 20.255$; $p < 0.001$) and lower peak amplitudes (Welch's t test: df = 72.38, t = -11.33, $p < 0.001$) (Figures 2B–2D), consistent with selection favoring their ability to evade detection by *O. ochracea*.[5] We also confirmed that root mean square amplitude, a measure of overall song amplitude, showed the same pattern (Figure S2).

Although female flies perceive sounds across a broad frequency range, their auditory sensitivity is sharply tuned to host frequencies between 4 and 6 kHz,[25] and a majority of small-wing males (ca. 70%) produced song >6 kHz. For those small-wing males with a carrier frequency overlapping the range of long-wing males (n = 9), averaged sound pressure level of their courtship song was approximately one-sixth that of long-wing males (59.27 vs. 75.27 dB) (Figures 2B and 2D). However, for those small-wing males whose carrier frequency shifted out of the typical range (n = 38), amplitude was far more variable, and a non-trivial fraction produced songs at or above the average long-wing peak amplitude of 75.27 dB (Figure 2B). Thus, despite carrier frequency shifts,

Table 1. Long-wing and small-wing morphology and courtship song characteristics

		Morphology			Song		
Sex	Morph	Sample size	Wing length (mm)	Pronotum length (mm)	Sample size	Peak amplitude (dB)	Carrier frequency (Hz)
Male	long-wing	166	12.91 ± 0.05	3.79 ± 0.02	38	75.27 ± 0.55	5,073 ± 37.22
	small-wing	177	9.10 ± 0.06	3.75 ± 0.02	47	63.64 ± 1.29	6,361 ± 113.58
Female	long-wing	254	12.36 ± 0.04	3.71 ± 0.01	–	–	–
	small-wing	56	8.51 ± 0.13	3.75 ± 0.02	–	–	–

Means ± standard errors of morphological differences in wing length, pronotum length, and courtship song differences quantified from those males that produced audible courtship songs during mating trials.

the resulting signal can be as loud or louder than typical male courtship song.

To put the morph differences into context, carrier frequency for long-wing males ranged from 4.68 to 5.42 kHz, a span of 0.74 kHz with a median of 5.13 kHz. By contrast, for small-wing males, it ranged from 4.85 to 8.38 kHz, a span of 3.53 kHz with a median of 6.83 kHz. Male crickets in the genus *Teleogryllus* typically sing in a narrow range of carrier frequencies; the frequency shift exhibited by small-wing *T. oceanicus* far exceeds the range of natural variation for normal, long-wing crickets in this species,[26] as well as its closely related sister species, which produce songs of 3.2 (*T. marini*) and 4.3 kHz (*T. commodus*).[27,28] Other *Teleogryllus* species also produce songs with carrier frequencies between 3.5 and 4.5 kHz, including *T. emma*, *T. taiwanemma*, *T. yezoemma*, and *T. mitratus*.[29,30]

We detected an interesting relationship between wing length and carrier frequency. As wing length increased, carrier frequency linearly decreased in small-wing males (Spearman rank correlation: $r = -0.39$, $p = 0.006$) but not in long-wing males (Spearman rank correlation: $r = -0.12$, $p = 0.455$) (Figure 2E). We checked that the former relationship remains significant when a potential outlier with wings ca. 6.6 mm is removed from the correlation test (Spearman rank correlation: $r = -0.35$, $p = 0.017$). The frequency modulation between chirp (the first set of pulses) and trill (the following longer set of pulses) components of the small-wing song shown in Figure 2A suggested that the relationship between carrier frequency and wing length in small-wing crickets could be attributable to a greater number of pulses in the chirps of individuals with longer wings, as opposed to mechanical properties of the wings themselves. We ruled out this behavioral explanation by contrasting the effects of wing length and pulse number on carrier frequency: wing length was significant (linear model: $F_{1,43} = 10.627$, $p = 0.002$), but pulse number was not (linear model: $F_{1,43} = 2.996$, $p = 0.091$). The greater variance and size-frequency scaling relationship in small-wing males is consistent with decanalization of spectral song properties in this novel morph.[31]

The immediate consequences of such a dramatic signal shift on the dynamics of mate recognition and choice are unknown. Crickets are typical of other taxa with acoustic mate recognition systems: female mate preference is predicted to match male song characteristics owing to their joint coevolution.[32–35] This association is a much-tested prediction of sexual selection models, yet despite some studies finding compelling evidence for a statistical association of male sexual trait values and female preferences,[36] the overall empirical evidence is equivocal.[37,38] To understand the behavioral dynamics permitting evolutionary persistence of such a dramatically diversified sexual signal, we next quantified female acceptance of courting males during mating trials with different morphs.

Females readily accept males with altered courtship songs

We tested whether audibly courting small-wing males face evolutionary disadvantages due to female rejection. Alternatively, females might exhibit a wide window of mate acceptance enabling the persistence of diversified song characters. Our data support the latter. Females accepted any male that produced an audible courtship song with similar probability, regardless of his wing morph (Figures 3A and 3B). Because we found no evidence for assortative mating, we analyzed 107 mating trials with long-wing and small-wing females and categorized them based on male morph and courtship behavior. First, we confirmed that females were more likely to mount males that produced an audible courtship song of any type than those that did not court the female (Fisher's exact test: $n = 102$, $p < 0.001$). Successful spermatophore transfer was similarly affected (Fisher's exact test: $n = 102$, $p < 0.001$), which is unsurprising because courtship song indicates that males are prepared to mate (Figure 3B). This increased mating success was similar for long-wing males (12.5% vs. 69.4% for non-courting vs. courting; Fisher's exact test: $n = 44$, $p = 0.005$) and small-wing males (50.0% vs. 80.0% for non-courting vs. courting; Fisher's exact test: $n = 58$, $p = 0.086$) (Figure 3B). In post hoc contrasts restricted to males that produced audible courtship songs, we found no difference in female mounting rate (Fisher's exact test: $n = 84$, $p = 0.386$) (Figure 3A; Table S1) or mating success (Fisher's exact test: $n = 86$, $p = 0.313$) (Figure 3B; Table S1). Over 90% of courting males were mounted irrespective of wing morph (Figures 3A and 3B), which closely matches findings that female *T. oceanicus* mount ca. 90% of singing males.[39] Although the probability of female mounting did not differ between the two male variants, females responded more quickly to courtship song produced by long-wing males (8.09 ± 2.08 s, mean ± SE) compared with small-wing males (14.14 ± 2.85 s, mean ± SE) (Wilcoxon rank-sum test, $W = 540.5$, $p = 0.036$) (Figure 3D; Table S1).

A minority of small-wing males stridulated but did not produce audible sound ($n = 5$), and some did not attempt courtship ($n = 10$). Unexpectedly, both groups elicited similar mounting rates from females as those that produced sound (Fisher's exact tests: inaudible courtship vs. audible courtship: $n = 53$, $p = 1.000$; no courtship attempt vs. audible courtship: $n = 58$, $p = 0.274$) (Figure 3A). This was not the case for long-wing males (Figure 3A), for whom

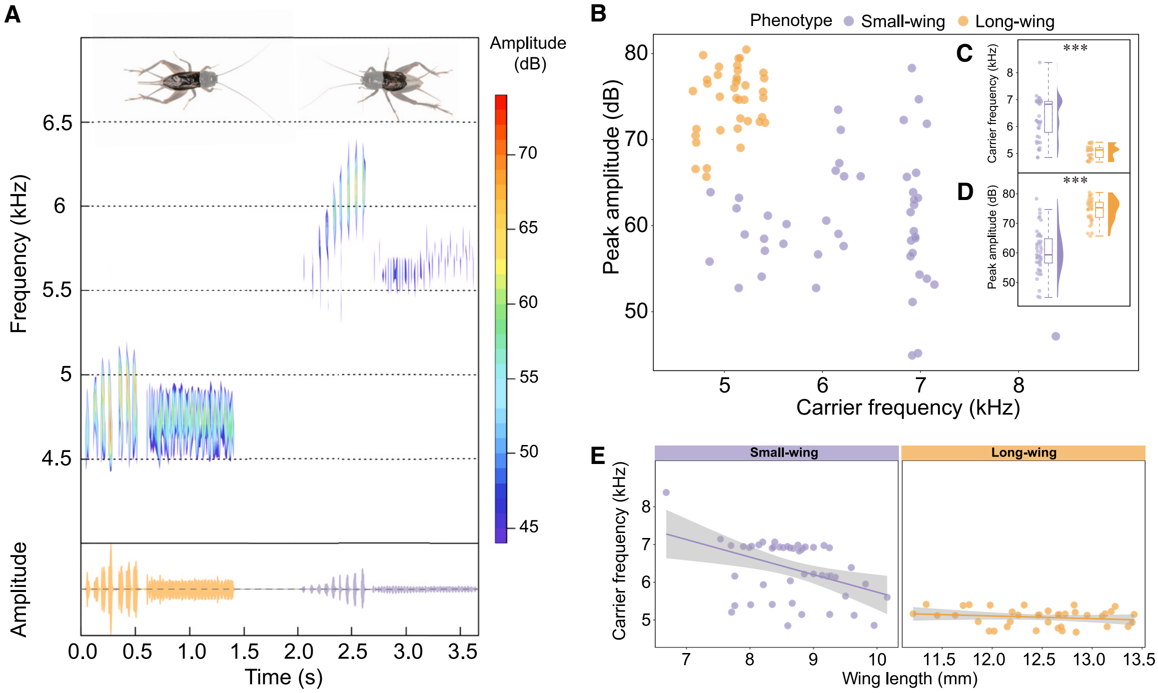


Figure 2. Altered male courtship song characteristics in small-wing *T. oceanicus*

(A) Representative long-wing (left) and audible small-wing (right) songs. Top panel shows carrier frequency over time for each song bout, with relative amplitude highlighted according to the heatmap on the right. Note the substantial frequency variation between small-wing song phrases, which is not present in long-wing song. Bottom panel shows the song structure with oscillograms. Example of audio recordings are provided (see Audio S1).

(B–D) (B) Morph comparisons for carrier frequency and peak amplitude. Audacity scaled relative peak amplitude was calibrated into approximately realistic sound pressure level from 10 cm away of signaler (see Figure S1). Purple and orange represent small-wing (n = 47) and long-wing (n = 38) observations, respectively. Medians (solid lines), inter-quartile ranges (boxes), and 1.5× inter-quartile range (whiskers) for carrier frequency (C) and peak amplitude (D) are shown inset with jittered points and half-eye plots. Statistically significant differences are indicated by asterisks; see main text for details. Comparisons between carrier frequency and root mean square amplitude are also provided (see Figure S2). Details are provided in the STAR Methods.

(E) Relationship between carrier frequency and forewing length for each morph, visualized using linear best-fit lines and shading indicating ±95% confidence intervals. This pattern was also tested with temporal variation of the number of pulses per chirp in small-wing but was not significant (see main text and Figure S3). Details are provided in the STAR Methods.

courtship increased female mounting rate (long-wing audible stridulation n = 36; long-wing no stridulation n = 8; Fisher's exact test: n = 44, p < 0.001). The latter is consistent with our prior work, which found that experimental ablation of long-wing male courtship song reduced female mounting by approximately half.[6,39] This morph difference should be interpreted cautiously due to small sample sizes, but despite courtship song's major influence on mounting, it may imply that small-wing males benefit more from short-range interaction with females, which may make satellite mating tactics more effective.[40–42] To explore this effect further, we performed a follow-up analysis restricted to males who produced audible courtship songs before being mounted by females. Among males mounted after producing audible courtship song, small-wing males were more likely to subsequently transfer sperm (ca. 90%) than long-wing males (ca. 70%) (Fisher's exact test: n = 79, p = 0.037) (Figure 3C).

Escaping loss during the initial stage of sexual character evolution

The evolution of sexual signal and female mate choice character shifts of large effect represent a chicken-and-egg problem: how does one change without initially being counter-selected by the other? For it may be the case that in the early stages of such a character shift, a novel male signal value that is rare in the population will compete against ancestral signal values that the overwhelming majority of females favor. Over a century of theoretical studies have examined spatial, genetic, and ecological conditions under which sexual traits such as signals and mate choice might shift to new optima and thereby contribute to reproductive isolation.[43,44] Most of these models rely on the implicit assumption that trait-preference covariance allows coordinated divergence—or diversification—of mate recognition systems,[1–3,45,46] which would lead to the prediction that substantially altered signal variants should be disfavored by females when rare.

However, *T. oceanicus* females readily accepted small-wing males with a broad and shifted range of signal frequencies as mates, despite the fact that frequency is a baseline filter during auditory processing and decision making in this genus.[47,48] This is surprising, given the expectation for tight tuning between sexually selected signal and receiver traits. Such broad acceptance prompts us to rule out several scenarios explaining the emergence of novel signals in this system. In the first, females retain ancestral frequency preferences and reject males that do not match these. This predicts that audible small-wing males

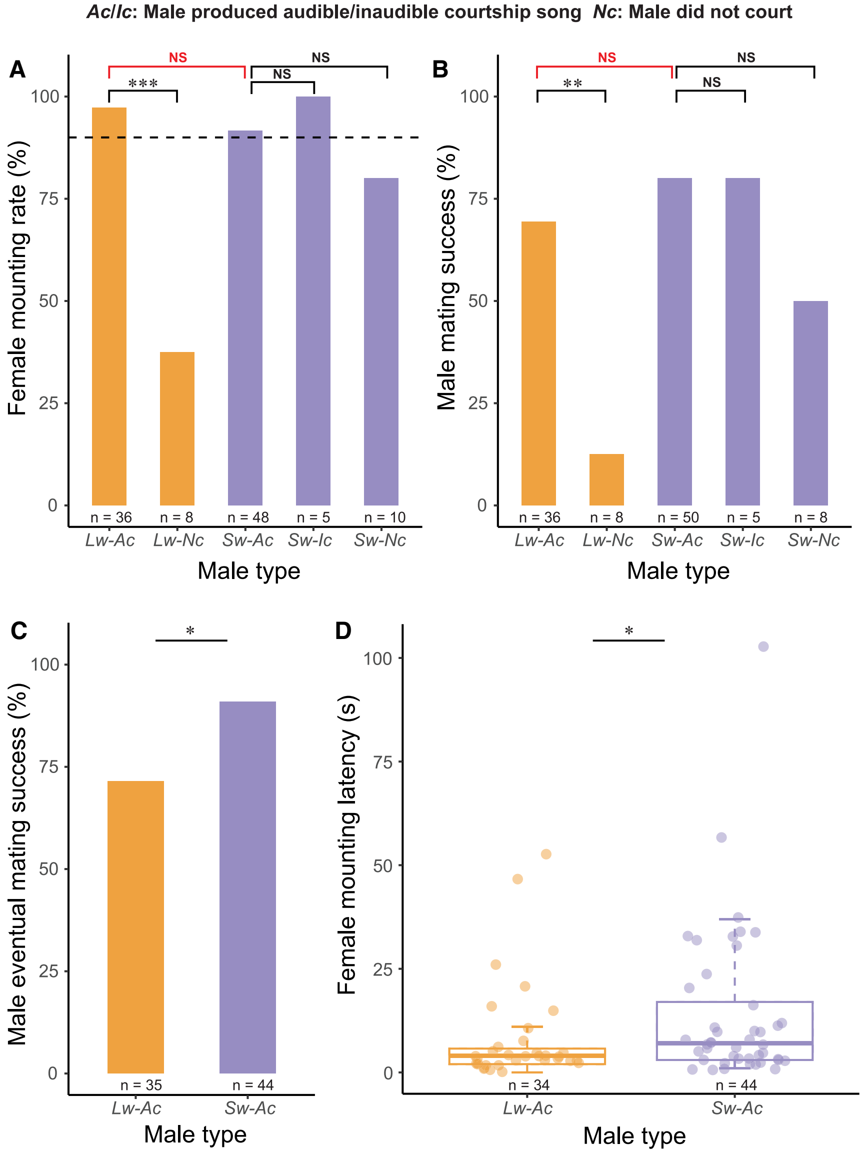


Figure 3. Female acceptance of males with altered courtship songs

(A and B) (A) Female mounting rate and (B) male mating success across 5 categories of courtship interaction: long-wing males that stridulate and produce audible song (*Lw-Ac*), long-wing males producing no stridulation thus no courtship song (*Lw-Nc*), small-wing males that stridulate and produce audible song (*Sw-Ac*), small-wing males that stridulate but do not produce audible song (*Sw-Ic*), and small-wing males producing no stridulation and thus no song (*Sw-Nc*). The dashed line in the former indicates a 90% mounting rate.

(C and D) (C) Eventual mating success for males that produced audible courtship song and were mounted by females, and (D) female mounting latency for trials where long-wing and small-wing males produced audible courtship song. Lines indicate medians, boxes inter-quartile ranges, and whiskers 1.5× inter-quartile ranges.

Asterisks indicate statistical significance; NS, not significant; see main text for details.

See also Table S1 and Videos S1, S2, and S3.

achieve lower mating rates, which we did not find. In the second, females evolve new courtship song preferences. This predicts that long-wing males achieve lower mating rates, which we did not find. Instead, our results support a scenario where permissive females enable establishment and spread of new sexual character traits.

Why are female *T. oceanicus* so accepting of mates that signal so far outside the normal range of male courtship song? Other recently reported *T. oceanicus* signal variants (“purring”) are less attractive to females compared with long-wing song.[49] Suggestively, *T. oceanicus* song has the highest carrier frequency of related congeners, females have an exceptionally broad acoustic sensitivity extending to ca. 100 kHz,[50] and the response of ascending auditory interneuron AN1, which enables song recognition in the brain, can be activated at frequencies above the normal song carrier frequency with sufficient amplitude.[51,52] However, behavioral responses switch from attraction to aversion at approximately 8–10 kHz due to auditory pathways that evolved under predation pressure from echolocating bats.[50] Thus, the conserved neurological basis of auditory processing in this species allows the perception of sounds that small-wing males make but likely constrains further upward frequency shifts.

One evolutionary driver underlying the female permissiveness we observed might be bottlenecks occurring during island colonization, which favor relaxed female preferences.[53] Another possibility is suggested by the observation that male song properties overlap less with female preferences in species parasitized by *O. ochracea*,[54–56] implying that acoustic signals may become uncoupled from receiver preferences when under selection from eavesdropping enemies. Such selective factors could ultimately prevent the evolutionary loss of new male signal variants by relaxing female mate discrimination. Irrespective of the ultimate causes of broad female acceptance thresholds, our findings support an under-appreciated mechanism by which initially rare sexual trait variants escape loss during their evolutionary origin. Mating permissiveness might be a widespread and general phenomenon during the critical initial phases of sexual signal diversification.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - ○ Lead contact

  - ○ Materials availability
  - ○ Data and code availability
- ● EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- ● METHOD DETAILS
  - ○ Mating experiment
  - ○ Morphological measurements
  - ○ Courtship song analysis
  - ○ Quantifying female acceptance of male courtship
- ● QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.11.063.

A video abstract is available at https://doi.org/10.1016/j.cub.2023.11.063#mmc7.

ACKNOWLEDGMENTS

We gratefully acknowledge funding from the UK Natural Environment Research Council to N.W.B. (NE/W001616/1) and to N.W.B. and J.G.R. (NE/T000619/1) and from the China Scholarship Council – University of St Andrews PhD scholarship to R.Z. (202004910423). We thank R. Tinghitella for sharing cricket stock when the COVID-19 pandemic precluded fieldwork; D. Forbes, M. McGunnigle, A. Grant, and A. Rosa for assisting with insect maintenance; and M. Ritchie, L. Yusuf, and three anonymous reviewers for insightful feedback on the manuscript.

AUTHOR CONTRIBUTIONS

R.Z., J.G.R., and N.W.B. conceived the project. R.Z., J.G.R., and N.W.B. designed the experiments. R.Z. collected data. R.Z. analyzed data. R.Z., J.G.R., and N.W.B. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: September 12, 2023
Revised: October 20, 2023
Accepted: November 29, 2023
Published: December 22, 2023

REFERENCES

1. Fisher, R.A. (1958). The Genetical Theory of Natural Selection, Second Edition (Dover Publications).
2. Kirkpatrick, M. (1982). Sexual selection and the evolution of female choice. Evolution *36*, 1–12.
3. Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA *78*, 3721–3725.
4. Bailey, N.W., and Moore, A.J. (2012). Runaway sexual selection without genetic correlations: social environments and flexible mate choice initiate and enhance the fisher process. Evolution *66*, 2674–2684.
5. Rayner, J.G., Aldridge, S., Montealegre-Z, F., and Bailey, N.W. (2019). A silent orchestra: convergent song loss in Hawaiian crickets is repeated, morphologically varied, and widespread. Ecology *100*, e02694.
6. Balakrishnan, R., and Pollack, G.S. (1996). Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. Anim. Behav. *51*, 353–366.
7. Rebar, D., Bailey, N.W., and Zuk, M. (2009). Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*. Behav. Ecol. *20*, 1307–1314.
8. Wagner, W.E., and Reiser, M.G. (2000). The importance of calling song and courtship song in female mate choice in the variable field cricket. Anim. Behav. *59*, 1219–1226.
9. Zuk, M., Rebar, D., and Scott, S.P. (2008). Courtship song is more variable than calling song in the field cricket *Teleogryllus oceanicus*. Anim. Behav. *76*, 1065–1071.
10. Panhuis, T.M., Butlin, R., Zuk, M., and Tregenza, T. (2001). Sexual selection and speciation. Trends Ecol. Evol. *16*, 364–371.
11. Ritchie, M.G. (2007). Sexual selection and speciation. Annu. Rev. Ecol. Evol. Syst. *38*, 79–102.
12. Broder, E.D., Elias, D.O., Rodríguez, R.L., Rosenthal, G.G., Seymoure, B.M., and Tinghitella, R.M. (2021). Evolutionary novelty in communication between the sexes. Biol. Lett. *17*, 20200733.
13. Rowland, W.J. (1989). Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). Behav. Ecol. Sociobiol. *24*, 433–438.
14. Moehring, A.J., and Boughman, J.W. (2019). Veiled preferences and cryptic female choice could underlie the origin of novel sexual traits. Biol. Lett. *15*, 20180878.
15. Alexander, R.D. (1962). Evolutionary change in cricket acoustical communication. Evolution *16*, 443–467.
16. Elliott, C.J.H., and Koch, U.T. (1985). The clockwork cricket. Naturwissenschaften *72*, 150–153.
17. Koch, U.T., Elliott, C.J.H., Schäffner, K.-H., and Kleindienst, H.-U. (1988). The mechanics of stridulation of the cricket *Gryllus campestris*. J. Comp. Physiol. *162*, 213–223.
18. Mhatre, N., Montealegre-Z, F., Balakrishnan, R., and Robert, D. (2012). Changing resonator geometry to boost sound power decouples size and song frequency in a small insect. Proc. Natl. Acad. Sci. USA *109*, E1444–E1452.
19. Zuk, M., Bailey, N.W., Gray, B., and Rotenberry, J.T. (2018). Sexual signal loss: the link between behaviour and rapid evolutionary dynamics in a field cricket. J. Anim. Ecol. *87*, 623–633.
20. Tanaka, S., Katagiri, C., Arai, T., and Nakamura, K. (2001). Continuous variation in wing length and flight musculature in a tropical field cricket, *Teleogryllus derelictus*: implications for the evolution of wing dimorphism. J. Entomol. Sci. *4*, 195–208.
21. Nasu, S., Imasaka, S., Suzuki, K., Ito, J., Hoshina, H., Kanno, H., Suyama, C., and Tokuda, M. (2019). Continuous variation in hind wing length of *Galerucella grisescens* (Coleoptera, Chrysomelidae, Galerucinae) and genetic basis of wing length determination. Appl. Entomol. Zool. *54*, 123–128.
22. Kemp, D.J. (2008). Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution *62*, 2346–2358.
23. Zhang, C.X., Brisson, J.A., and Xu, H.J. (2019). Molecular mechanisms of wing polymorphism in insects. Annu. Rev. Entomol. *64*, 297–314.
24. Roff, D.A. (1994). Why is there so much genetic variation for wing dimorphism? Popul. Ecol. *36*, 145–150.
25. Robert, D., Amoroso, J., and Hoy, R.R. (1992). The evolutionary convergence of hearing in a parasitoid fly and its cricket host. Science *258*, 1135–1137.
26. Pascoal, S., Mendrok, M., Wilson, A.J., Hunt, J., and Bailey, N.W. (2017). Sexual selection and population divergence II. Divergence in different sexual traits and signal modalities in field crickets (*Teleogryllus oceanicus*). Evolution *71*, 1614–1626.
27. Moran, P.A., Hunt, J., Mitchell, C., Ritchie, M.G., and Bailey, N.W. (2020). Sexual selection and population divergence III: interspecific and intraspecific variation in mating signals. J. Evol. Biol. *33*, 990–1005.
28. Otte, D., and Alexander, R.D. (1983). The Australian crickets (Orthoptera: Gryllidae) (Academy of Natural Sciences of Philadelphia).
29. Baker, E., Price, B.W., Rycroft, S.D., Hill, J., and Smith, V.S. (2015). BioAcoustica: a free and open repository and analysis platform for bioacoustics. Database *2015*, bav054.

30. Honda-Sumi, E. (2005). Difference in calling song of three field crickets of the genus *Teleogryllus*: the role in premating isolation. Anim. Behav. *69*, 881–889.
31. Bailey, N.W., Pascoal, S., and Montealegre-Z, F. (2019). Testing the role of trait reversal in evolutionary diversification using song loss in wild crickets. Proc. Natl. Acad. Sci. USA *116*, 8941–8949.
32. Gray, D.A., and Cade, W.H. (2000). Sexual selection and speciation in field crickets. Proc. Natl. Acad. Sci. USA *97*, 14449–14454.
33. Gray, D.A., and Cade, W.H. (1999). Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. Evolution *53*, 848–854.
34. Ritchie, M.G. (1996). The shape of female mating preferences. Proc. Natl. Acad. Sci. USA *93*, 14628–14631.
35. Rodríguez, R.L., Ramaswamy, K., and Cocroft, R.B. (2006). Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. Proc. Biol. Sci. *273*, 2585–2593.
36. Shaw, K.L., and Lesnick, S.C. (2009). Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. Proc. Natl. Acad. Sci. USA *106*, 9737–9742.
37. Greenfield, M.D., Alem, S., Limousin, D., and Bailey, N.W. (2014). The dilemma of Fisherian sexual selection: mate choice for indirect benefits despite rarity and overall weakness of trait-preference genetic correlation. Evolution *68*, 3524–3536.
38. Fowler-Finn, K.D., and Rodríguez, R.L. (2016). The causes of variation in the presence of genetic covariance between sexual traits and preferences. Biol. Rev. Camb. Philos. Soc. *91*, 498–510.
39. Bailey, N.W., McNabb, J.R., and Zuk, M. (2008). Preexisting behavior facilitated the loss of a sexual signal in the field cricket *Teleogryllus oceanicus*. Behav. Ecol. *19*, 202–207.
40. Balakrishnan, R., and Pollack, G.S. (1997). The role of antennal sensory cues in female responses to courting males in the cricket *Teleogryllus oceanicus*. J. Exp. Biol. *200*, 511–522.
41. Steiger, S., Ower, G.D., Stökl, J., Mitchell, C., Hunt, J., and Sakaluk, S.K. (2013). Sexual selection on cuticular hydrocarbons of male sagebrush crickets in the wild. Proc. Biol. Sci. *280*, 20132353.
42. Tregenza, T., and Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. Anim. Behav. *54*, 979–984.
43. Lande, R. (1982). Rapid origin of sexual isolation and character divergence in a cline. Evolution *36*, 213–223.
44. Ispolatov, J., and Doebeli, M. (2009). Diversification along environmental gradients in spatially structured populations. Evol. Ecol. Res. *11*, 295–304.
45. Fisher, R.A. (1915). The evolution of sexual preference. Eugen. Rev. *7*, 184–192.
46. Prum, R.O. (2010). The Lande–Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. Evolution *64*, 3085–3100.
47. Kostarakos, K., Hennig, M.R., and Römer, H. (2009). Two matched filters and the evolution of mating signals in four species of cricket. Front. Zool. *6*, 22.
48. Bailey, N.W., Moran, P.A., and Hennig, R.M. (2017). Divergent mechanisms of acoustic mate recognition between closely related field cricket species (*Teleogryllus* spp.). Anim. Behav. *130*, 17–25.
49. Fitzgerald, S.L., Anner, S.C., and Tinghitella, R.M. (2022). Varied female and male courtship behavior facilitated the evolution of a novel sexual signal. Behav. Ecol. *33*, 859–867.
50. Hoy, R.R., Pollack, G.S., and Moiseff, A. (1982). Species-recognition in the field cricket, *Teleogryllus oceanicus*: behavioral and neural mechanisms. Am. Zool. *22*, 597–607.
51. Schöneich, S. (2020). Neuroethology of acoustic communication in field crickets - from signal generation to song recognition in an insect brain. Prog. Neurobiol. *194*, 101882.
52. Hedwig, B.G. (2016). Sequential filtering processes shape feature detection in crickets: a framework for song pattern recognition. Front. Physiol. *7*, 46.
53. Tinghitella, R.M., and Zuk, M. (2009). Asymmetric mating preferences accommodated the rapid evolutionary loss of a sexual signal. Evolution *63*, 2087–2098.
54. Blankers, T., Hennig, R.M., and Gray, D.A. (2015). Conservation of multivariate female preference functions and preference mechanisms in three species of trilling field crickets. J. Evol. Biol. *28*, 630–641.
55. Dobbs, O.L., Talavera, J.B., Rossi, S.M., Menjivar, S., and Gray, D.A. (2020). Signaler–receiver–eavesdropper: risks and rewards of variation in the dominant frequency of male cricket calls. Ecol. Evol. *10*, 12364–12371.
56. Gray, D.A., Gabel, E., Blankers, T., and Hennig, R.M. (2016). Multivariate female preference tests reveal latent perceptual biases. Proc. Biol. Sci. *283*, 20161972.
57. R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.
58. Sarmiento-Ponce, E.J., Rogers, S., and Hedwig, B. (2021). Does the choosiness of female crickets change as they age? J. Exp. Biol. *224*, jeb241802.
59. Bailey, N.W. (2011). Mate choice plasticity in the field cricket *Teleogryllus oceanicus*: effects of social experience in multiple modalities. Behav. Ecol. Sociobiol. *65*, 2269–2278.
60. Bailey, N.W. (2008). Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. Behav. Ecol. *19*, 960–966.
61. Simmons, L.W., Thomas, M.L., Simmons, F.W., and Zuk, M. (2013). Female preferences for acoustic and olfactory signals during courtship: male crickets send multiple messages. Behav. Ecol. *24*, 1099–1107.
62. Sueur, J., Aubin, T., and Simonis, C. (2008). Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics *18*, 213–226.
63. Schneider, W.T., Rutz, C., Hedwig, B., and Bailey, N.W. (2018). Vestigial singing behaviour persists after the evolutionary loss of song in crickets. Biol. Lett. *14*, 201.70654.
64. Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis, Second Edition (Springer Cham).
65. Kassambara, A. (2023). ggpubr: “ggplot2” Based Publication Ready Plots. The Comprehensive R Archive Network.
66. Aphalo, P.J. (2023). ggpmisc: Miscellaneous Extensions to “ggplot2”. The Comprehensive R Archive Network.
67. Hartig, F. (2022). Dharma: Residual Diagnostics for Hierarchical (Multi-level/Mixed) Regression Models. R Package, Ver. 0.4.6. http://florianhartig.github.io/DHARMa/.
68. Venables, W.N., and Ripley, B.D. (2002). Modern Applied Statistics with S, Fourth Edition (Springer).
69. Fox, J., and Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition (Sage Publications).
70. Mangiafico, S.S. (2023). rcompanion: Functions to Support Extension Education Program Evaluation (Rutgers Cooperative Extension). The Comprehensive R Archive Network.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Analyzed data	This study	https://doi.org/10.17630/5b31ee44-0eba-4d55-a620-6bee93ebeac9
R code	This study	https://doi.org/10.17630/5b31ee44-0eba-4d55-a620-6bee93ebeac9
Experimental models: Organisms/strains		
Teleogryllus oceanicus	University of Hilo, Hawaii	Renjie Zhang, Jack G. Rayner and Nathan W. Bailey
Software and algorithms		
R	R Core Team[57]	https://www.r-project.org/
Audacity	v.3.3.2	https://www.audacityteam.org/
Show-amplitude	Plug-in	https://forum.audacityteam.org/t/peak-amplitude-analyze-plug-in/31723
Adobe Premiere Pro	2022	https://www.adobe.com/uk/products/premiere.html
VLC	v.3.0.18	https://www.videolan.org/
Other		
SLR camera	Nikon	Nikon D3300
Sound level meter	Shenzhen, China	CEM-DT-805
Laptop	Apple	MacBook Air 2020 (M1 chip)
Mobile phone	Apple	iPhone 11

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact on reasonable request, Renjie Zhang (rz40@st-andrews.ac.uk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Analyzed data supporting the current research are publicly available from the Online supplemental information and the University of St Andrews PURE Research Information System repository as of the date of publication. The DOI is listed in the key resources table.
- All original code has been deposited at the University of St Andrews PURE Research Information System repository and is publicly available as of the date of publication. The DOI is listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

A laboratory population of *Teleogryllus oceanicus* containing both long-wing and small-wing individuals was derived from a wild population in 2020 at the University of Hilo, Hawaii. As the small-wing phenotype was initially rare (~ 10%), we bred a pair with a small-wing father and subsequently used the resulting stock. Crickets were kept in translucent, 20 L plastic tubs at 28 °C, on a photo-reversed 12:12 light/dark cycle. Burgess Excel Junior and Dwarf rabbit pellets were provided *ad libitum* and each container contained cardboard egg cartons for shelter. All boxes were maintained in the same walk-in incubator and maintained twice weekly.

METHOD DETAILS

Mating experiment

Virgins were used in mating experiments, which we ensured by isolating them during their final instar. Individuals were maintained in 113 mL deli pots on twice weekly basis with food, egg carton and a small cotton-plugged glass water vial. Each subject was checked daily for adult eclosion. We haphazardly paired 126 males and 126 females in four combinations for mating trials (morphs coded male morph—female morph): long-wing—long-wing (n = 49), long-wing—small-wing (n = 4), small-wing—long-wing (n = 67) and small-wing—small-wing (n = 6). Crosses involving small-wing females were limited by their rarity. To ensure sexual maturity, we tested males 7 - 15 days and females 7 - 9 days post adult eclosion. There is equivocal evidence about whether female discrimination decreases with age,[58] but we used a younger and narrower range of female ages to reduce the possibility that results could be affected by this. This age range of virgin females, and the broader mate trial conditions, are consistent with the considerable body of prior work on female mate choice in this species; providing confidence in our ability to detect discrimination if it is expressed.[59,60] All trials were conducted during the crickets' dark cycle at 28 ± 1.5 °C under dim red light.

Following established procedures in *T. oceanicus*,[59,60] mating trials were performed in translucent boxes (17 × 11× 5 cm) and filmed using the video function on a Nikon D3300 single-lens reflex camera fixed ca. 40 cm above the container. Video settings were: F 5.6, iso 800, resolution 1920x1080 (50 fps), audio sampling rate 48,000 Hz. The male and female were placed at standardized positions (left and right, respectively) under a 113 mL deli pot to acclimate for 2-minutes. Then, we started filming and released the crickets. A clear plastic sheet covered the container to prevent crickets from escaping. Each trial lasted 15 minutes or once male spermatophore transfer was completed. Between trials, the experiment arena was cleaned with 75% ethanol to remove odour cues. We alternated trials between long-wing and small-wing males. Average pronotum length was obtained from 3 measurements. We anecdotally noted striking forewing asymmetry in small-wing individuals, so both left and right wings were measured, and averaged for the purposes of some data displays. In long-wing individuals, we measured the right forewing which is folded at rest atop the left forewing. All measurements were obtained to the nearest 0.01 mm using a digital vernier calliper (RTP 6'', Rowland Tools). All crickets were weighed to the nearest 0.001g within 3 days using a digital scale (BDH AL-300). As pronotum and wing length are fixed after adult eclosion, these parameters were measured within one fortnight.

Morphological measurements

We measured right forewing length and pronotum length to the nearest 0.01 mm of 653 individuals (254 long-wing females, 166 long-wing males, 56 small-wing females, 177 small-wing males), of both morphs and sexes, across two generations. This set of individuals included those males and females used in mating trials above. To ensure consistency for morphological analysis, only right forewing length and pronotum length measured from the first time were used.

Courtship song analysis

We inspected videos using VLC v.3.0.18 and extracted uncompressed .wav files using Adobe Premiere Pro 2022 (sampling rate: 48,000 Hz; resolution: 16-bt). Male song frequency has been found to be highly repeatable both within and across trials.[7] To cover the range of phenotypic variation present across all males, we included in our analysis songs of short duration, for example those which included only several complete song phrases, as these were accepted by females, as well as cases where males produced more than one bout of courtship song.

We measured carrier frequency and scaled relative peak amplitude using Audacity v.3.3.2. First, to reduce background noise, all song files were bandpass-filtered below 3.5 kHz[61] using the function "High-pass filter" with a roll-off value of 48 dB. Second, for those males who produced multiple bouts of courtship song, we selected three courtship song phrases and output them as a new song dataset; these comprised the one song phrase with the highest amplitude for that trial, plus the two song adjacent song phrases. If there were insufficient adjacent song phrases, one or two courtship song phrases were haphazardly selected from the same trial. We estimated carrier frequency using the function "Plot Spectrum", relative peak amplitude using the plug-in "Show-amplitude", and root mean square amplitude using the function "Measure RMS". Pulse number per chirp was counted visually and averaged. Fifteen ".wav" files were excluded from analysis due to insufficient song phrases (long-wing: n = 7, small-wing: n = 8). In total, we analysed 85 courtship songs (long-wing: n = 38, small-wing: n = 47).

Audio recordings extracted from videos produced scaled relative peak amplitudes only. Because recording conditions were standardised with constant gain settings, we were able to convert relative dB levels evaluated in Audacity to a reasonable approximation of absolute sound pressure. We constructed a calibration curve (see Figure S1) by playing back a standard long-wing courtship song recording in the centre of the arena over 15 dB increments spanning a natural range of possible sound levels. We recorded the song using the same digital camera under the experimental settings above, i.e. with the plastic sheet atop the arena. Then we took the plastic sheet off and measured the peak amplitude approximately 10 cm from the speaker with a digital sound-level meter (CEM-DT-805, Shenzhen, China). We measured dB levels three times for each amplitude increment and averaged them using the function "meandB" from the Seewave R package.[62] Then, we performed a linear regression of peak amplitude measured by the sound level meter on scaled relative peak amplitude from Audacity to derive a conversion formula: ***Sound level meter absolute peak amplitude* = 87.5 + 0.949 ×** ***Audacity scaled relative peak amplitude***. This calibration produced a high linear $R^2 = 0.95$ (see Figure S1), allowing us to estimate song sound level experienced at a distance of 10 cm. Any effect of placing a lid over the arena on frequency measurements is expected to be trivial owing to the lid's thinness (ca. 0.5 cm) relative to the wavelength of *T. oceanicus* carrier frequency (ca. 7.0 cm). We confirmed this visually by comparing frequency spectrum with vs. without a lid of a single recording. Average and standard error of peak amplitude were calculated using the output from the function "meandB" and "sddB" in the Seewave R package.[62]

Two separate high-quality recordings of male courtship song were taken from representative long-wing and small-wing males (randomly selected from the offspring of mating trials) at 25 °C in a sound-proof room under dim light. Audio files were recorded using

Macbook Air 2020 (M1 chip) Speakers via Audacity. The recording sampling rate was set at 44,100 Hz with a 16-bit resolution and data were exported as .wav files. We filtered the background noise as described in above, and selected one complete song with the highest amplitude for each male phenotype using Audacity (ca. 1.5 s for each song). Long-wing and small-wing songs were combined into the same .wav file (Lw: 0 - 1.4 s, Sw: 2 - 3.6 s; see Audio S1) for spectrogram visualization. Then, a spectrogram was drawn using the function "Spectro" from the Seewave R package.[62] To better visualize the structural differences and frequency changes corresponding to each part of the male courtship song, a time-scaled waveform was plotted. Amplitude values were normalized as recommended by the package developer. Other parameters were set to the default: window name as "hanning", and window length as 512.

Quantifying female acceptance of male courtship

We quantified whether females *mounted* males at least once during the trial (a binary measure) and, if they did, whether the male successfully *mated*, which we inferred from the presence of a transferred spermatophore (also a binary measure). Several data exclusions defined *a priori* were necessary to avoid confounds: (1) if females made thermoregulation movements characterised by elevation of the forewings and fluttering of the hindwings (n = 17); (2) if there was no physical contact between male and female (n = 1); (3) if the male wing was damaged (n = 1). We retained 40 long-wing—long-wing, 4 long-wing—small-wing, 57 small-wing—long-wing, and 6 small-wing—small-wing trials for further analysis.

Males do not always court females even when a pair have made physical contact and the opportunity is available. In addition, wing movements associated with song in the present experiment were not always accompanied by audible sound (see Video S3). In such cases, it can be inferred that the small-wing male was receptive for mating despite not producing an audible signal, similar to silent wing-movement behaviour documented in flatwing *T. oceanicus* males.[63] As such, we classified courtship trial outcomes into 5 categories according to the wing movements associated with courtship song and the acoustic signal itself: (1) long-wing males that did produce courtship song (*Lw-Ac*, n = 36) (see Video S1); (2) long-wing males that did not produce a courtship song (*Lw-Nc*, n = 8); (3) small-wing males that produced audible courtship song (*Sw-Ac*, n = 48) (see Video S2); (4) small-wing males that produced wing movements associated with courtship song but which were inaudible; i.e., there was no unique frequency component above the baseline detected from 4 to 8 kHz (*Sw-Ic*, n = 5); In one trial, the male produced certain phrases of the song (i.e. trills) with extremely low amplitude by adjusting his wing angle. An example of this phenomenon can be viewed in the Video S3. (5) small-wing males that did not produce wing movements associated with courtship song (*Sw-Nc*, n = 10), of which two individuals produced audible courtship song after the first mount and were then re-classified into *Sw-Ac* for male mating success analysis.

In trials where females mounted males after males had produced an audible courtship song (35 long-wing, 44 small-wing), we recorded female *latency to mount* to the nearest second as the duration after the onset of male courtship. Female mounting was recorded if the female remained atop of the male's back for at least 2 seconds. As there was no obvious evidence for morph-specific assortative mating between long-wing and small-wing phenotypes, we included trials with any female type. One trial with a long-wing male was excluded from analysis as the interval between his first and second singing attempt was greater than 3 minutes.

QUANTIFICATION AND STATISTICAL ANALYSIS

All analyses were performed in R v.4.3.0[57] and data were visualized with the ggplot2 R package.[64] Residuals from initial analyses were evaluated using Q-Q and density plots in ggpubr R package[65] and assessed for normality using Shapiro-Wilk tests. We used Welch's t-tests (two-tailed) to compare wing and pronotum length between morphs of each sex, and peak amplitude of male courtship song. A Levene's test was used to test homogeneity of variances in carrier frequency of courtship song between male morphs. Morph variation in slopes of pronotum length and wing length was compared using linear models fitted with two-way interaction terms involving morph and the factor of interest. Wilcoxon rank-sum tests were used to examine carrier frequency of male courtship song and female mounting latency. Spearman rank correlations were used to examine relationships between carrier frequency and wing length. A multiple linear regression model was used to test effects of wing length and pulse number on carrier frequency of small-wing courtship song. The R package ggpmisc was used to calculate R^2 and formula for fitted linear models.[66]

Fisher's Exact tests were used to analyse female acceptance (mounting and mating) during mating trials between all audibly courting males and non-courting males, for *post hoc* contrasts between audibly courting long-wing and small-wing males, and for evaluating the impact of male courtship on female mounting and male mating. Separate generalized liner models (GLMs) with binomial distributions were used to compare mounting and mating success between long-wing and small-wing males that produced audible courtship song. For these, we included male age and pronotum length of both sexes as covariates. Two trials were excluded from GLM analysis of mounting, mating success and female latency to mount due to missing female body size values. The same long-wing male outlier was removed from GLM analysis for female mounting latency as mentioned in above section. We detected overdispersion in female mating latency data using the R package DHARMa,[67] so a GLM with negative binomial distribution was applied using the R package MASS[68] and the same covariates as above. Significance testing was performed using chi-square tests with type II sums of squares in the R package car.[69] McFadden's pseudo R^2 was calculated via the "nagelkerke" function from the R package rcompanion.[70]