

Repeated convergent loss of cricket song

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3 A silent orchestra: convergent song loss in Hawaiian crickets

4 is repeated, morphologically varied, and widespread

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19 Main text

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21 Host-parasite interactions are predicted to drive the evolution of defences and counter-
22 defences, but the ability of either partner to adapt depends on new and advantageous traits
23 arising. The loss of male song in Hawaiian field crickets (*Teleogryllus oceanicus*) subject to
24 fatal parasitism by eavesdropping flies (*Ormia ochracea*) is a textbook example of rapid
25 evolution in one such arms race (Dugatkin 2008). Male crickets ordinarily sing to attract
26 females by rubbing their forewings together, which produces sound by exciting acoustic
27 resonating structures formed from modified wing veins ('normal-wing', Nw: Fig. 1A). The
28 resulting song is the target of strong sexual selection by conspecific females. However, in
29 Hawaii, male song also attracts female flies that squirt larvae onto males or nearby female
30 crickets; the larvae then burrow into, consume, and ultimately kill the host. The flies thus
31 impose strong natural selection on male song.

32 Approximately 15 years ago, Zuk et al. (2006) observed the emergence and rapid
33 spread of silent male mutant *T. oceanicus* phenotypes in parasitized populations on Kauai and
34 Oahu. Song loss is caused by genetic mutations that greatly reduce or eliminate sound-
35 producing structures by superficially feminising male wing venation ('flatwing', Fw; Fig.
36 1A) – all females have unmodified wings, and are incapable of producing song. Due to its
37 protective effect against the parasitoid fly, the flatwing phenotype spread very rapidly (Zuk et
38 al. 2006). However, flatwing phenotypes are associated with independent genetic
39 architectures on Kauai and Oahu, providing a striking example of convergent evolution on a
40 contemporary timescale (Pascoal et al. 2014). On visits to parasitized cricket populations in
41 2017 and 2018, we discovered two additional wing phenotypes – 'small-wing' (Sw: Fig. 1B)
42 and 'curly-wing' (Cw: Fig. 1B). Small-wing and curly-wing differ noticeably from flatwing

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43 and from each other, but they all eliminate or severely reduce the acoustic signals that are
44 attractive to flies.

45 We discovered these reduced-song phenotypes while performing transect surveys in
46 parasitized populations where flatwing morphs have not come to predominate (Fig. 2A). We
47 first identified curly-wing in the ‘CC’ population (initials refer to site codes; Fig. 2A) in
48 2017, and name it for its similarity with the *Drosophila* wing mutation described nearly a
49 century ago by Ward (1923). To our knowledge, it has never been described in crickets. In
50 lab populations reared from eggs of ca. 30 wild-caught females, curly-wing morphology
51 persisted across four generations at similar proportions (~50%), strongly suggesting a
52 heritable basis. The trait is observable immediately upon adult eclosion, and other lab
53 populations reared in the same growth chamber do not express it.

54 Curly-wing morphology definitively protects calling males from parasitoid attack
55 relative to typical Nw males (Fig. 2B,C). First, we found that males with Nw venation, but
56 exhibiting curly-wing morphology like that shown in Fig. 1C, do not sing as loudly as typical
57 Nw males (Wilcox rank sum test: N=15, P<0.001; Fig 2B; comparing songs measured in the
58 lab using a CEM DT-805 sound level meter 5 cm from test subjects). Flatwing males also
59 attempt to sing (Schneider et al. 2018), but the amplitude of acoustic stimuli produced during
60 wing movement did not differ between CwNw and Fw males (N=13, P=1.000). Like Fw
61 males, CwNw males produced variable, but lower-amplitude, peak frequencies (Fig. 2B).
62 Second, we found that the sound reduction caused by curly-wing morphology prevents *O.*
63 *ochracea* attack (Fig. 2C). We performed playback trials at the CC site using looped calling
64 songs recorded in the lab at 25±1°C from 4 Nw and 4 CwNw males. Songs were played on
65 SanDisk Mp3 players through Sony SRS-m30 speakers underneath fly traps (modified 1.5L
66 plastic bottles with the funnel-end inverted), broadcast at their originally-recorded volumes.
67 Since Cw males were only found in populations that also contained calling Nw males (Fig.

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68 2A), we designed playbacks to mimic natural conditions by placing three traps 11m apart in a
69 triangle: one typical Nw song, one CwNw song, and a third without playback as a negative
70 control. Trials lasted 5 minutes and were performed in dry weather between sunset (~6.10pm)
71 and 8.30pm when the fly is active (Beckers & Wagner 2012). All pairings of typical Nw and
72 CwNw song models were repeatedly tested over 4 nights and rotated among speakers
73 between trials. Like the negative controls, CwNw songs never resulted in a fly entering the
74 trap, whereas typical Nw songs attracted flies in 28.13% of trials (paired Wilcoxon signed
75 rank test: N=64, P<0.001).

76 In the same field season, when surveying a different parasitised population of
77 Hawaiian *T. oceanicus* ('UH' in Fig. 2A) in which less than 5% of males exhibit flatwing
78 morphology, we noted a substantial proportion of males (N=28, 27.18%) with unusually
79 small, but normally-veined forewings ('SwNw', Fig. 1B). We temporarily removed 12
80 SwNw males from the field and measured courtship song that they produced when exposed
81 to females (mean = 61.83 dB \pm 2.99 SE, see supplementary videos). Two of the 12 produced
82 acoustic stimuli below the recordable atmospheric noise level of ca. 45 dB, so we
83 conservatively dummy-coded these in analyses as producing song at 45 dB. One of the 12
84 had forewings of differing lengths and sang at up to 80 dB, towards the lower end of the
85 normal range (Balakrishnan & Pollack 1996), but this was the exception. The other 11
86 produced acoustic signals at substantially lower than normal levels. These observations
87 strongly suggest that their reduced song amplitude also protects SwNw males against
88 parasitoid attack.

89 We observed that small-wing morphology not only affected crickets' forewings,
90 which males use to produce song, but also the hindwings, which both sexes use for flight
91 (Fig. 1B). Brachyptery is commonly observed in the hindwings only of related species
92 (though not, to our knowledge, in *T. oceanicus*) and is highly heritable in related species

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93 (Roff 1994). An important distinction is that brachypterous forms of field crickets such as
94 *Gryllus firmus* gain a fitness advantage by divesting energy from maintaining large
95 hindwings and flight muscle, while boosting their attractiveness to females through increased
96 calling effort using the forewings (Crnokrak & Roff 1998). In *T. oceanicus*, small-wing
97 males are unable to produce ordinary calling or courtship song, owing to their reduced
98 forewings, and so would gain no such benefit.

99 The initial discovery of flatwing stimulated research into behavioural and
100 physiological consequences of trait loss and rapid evolution, and a population of *T. oceanicus*
101 was recently described on Molokai in which flatwing males produce severely attenuated,
102 broad-band acoustic stimuli (Tinghitella et al. 2018). Our identification of additional
103 protective, reduced-song wing morphs raises many questions. The emergence of alternative
104 adaptive phenotypes may have inhibited the spread of flatwing males and could account for
105 their variable proportions observed in different populations (Zuk et al. 2018). Do flatwing,
106 curly-wing and small-wing males differ in attractiveness to females, and does one phenotype
107 have an advantage over others? They all appear capable of co-expression in the same male,
108 and females also express curly-wing and small-wing, so it will be important to dissect their
109 genetic architecture.

110 The recurrent adaptive loss of song across small, fragmented populations of Hawaiian
111 field crickets illustrates the multiple, morphologically varied routes by which this male trait
112 can be functionally lost. Our observations are consistent with recent evidence for high
113 evolvability of trait loss under negative selection (Xie et al. 2018), a phenomenon widely
114 observed among costly sexually selected traits (Wiens 2001), and which may play an
115 important role in rapid adaptation of populations to novel environments or selection
116 pressures. The recurrent disappearance of song in *T. oceanicus* suggests evolutionary trait or

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117 signal loss could be a common means for hosts to evade their parasites, owing to the fitness
118 advantages that arise from evading detection.

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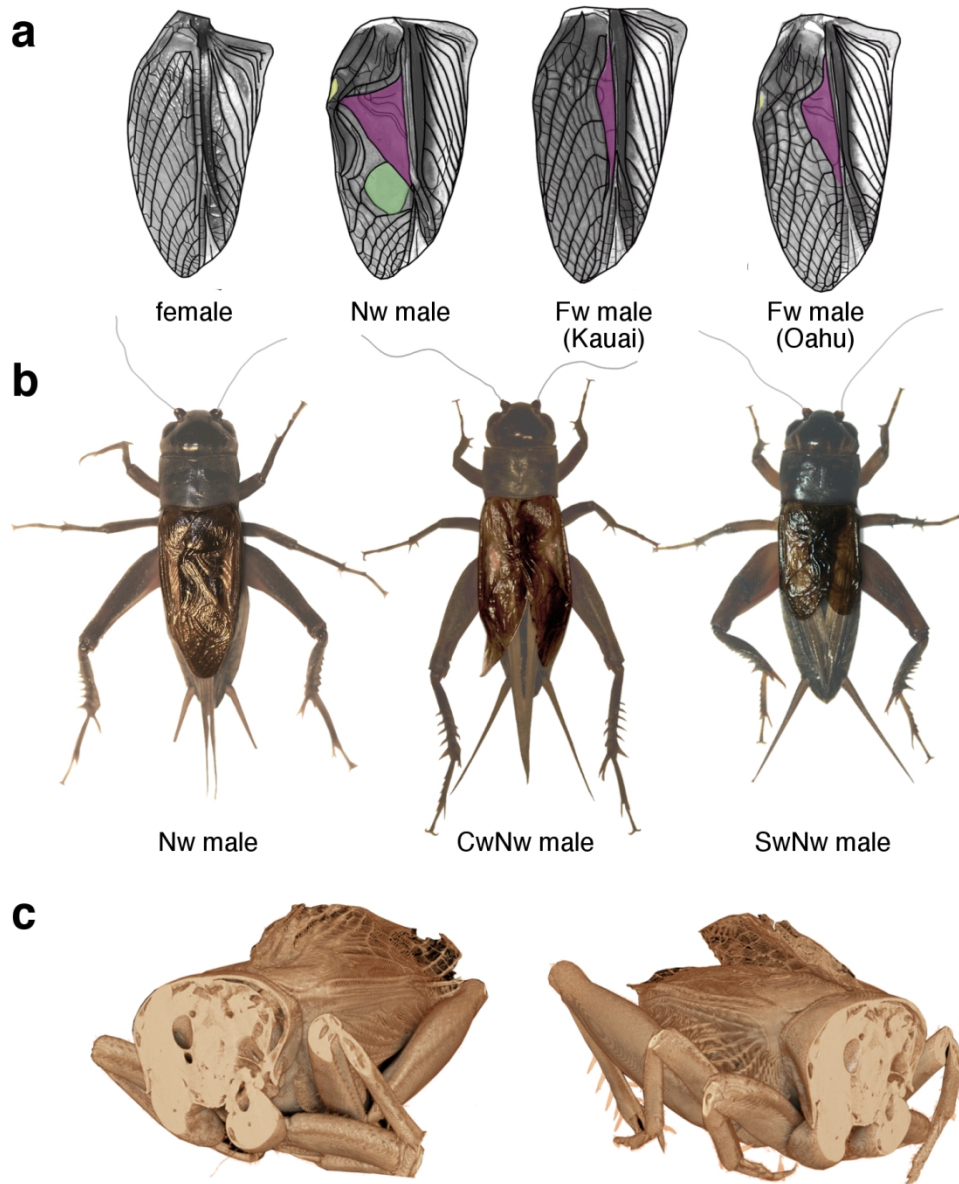


Figure 1. Alternative male-silencing wing morphs of Hawaiian *T. oceanicus*. (A) Venation variants: traced micrographs showing forewing venation patterns (adapted from Pascoal et al. 2014) of a female and Nw male, and Fw males from the different islands, with sound-producing structures highlighted (purple='harp', green='mirror', yellow='plectrum'). (B) Newly described shape and size variants: typical Nw male (left) alongside CwNw male and SwNw male; note that both forewings and hindwings are reduced. (C) Micro-CT scans of a CwFw male with forewings in resting position and head and thorax omitted, showing how marginal wing surfaces 'peel up' and preclude physical engagement during wing movement.

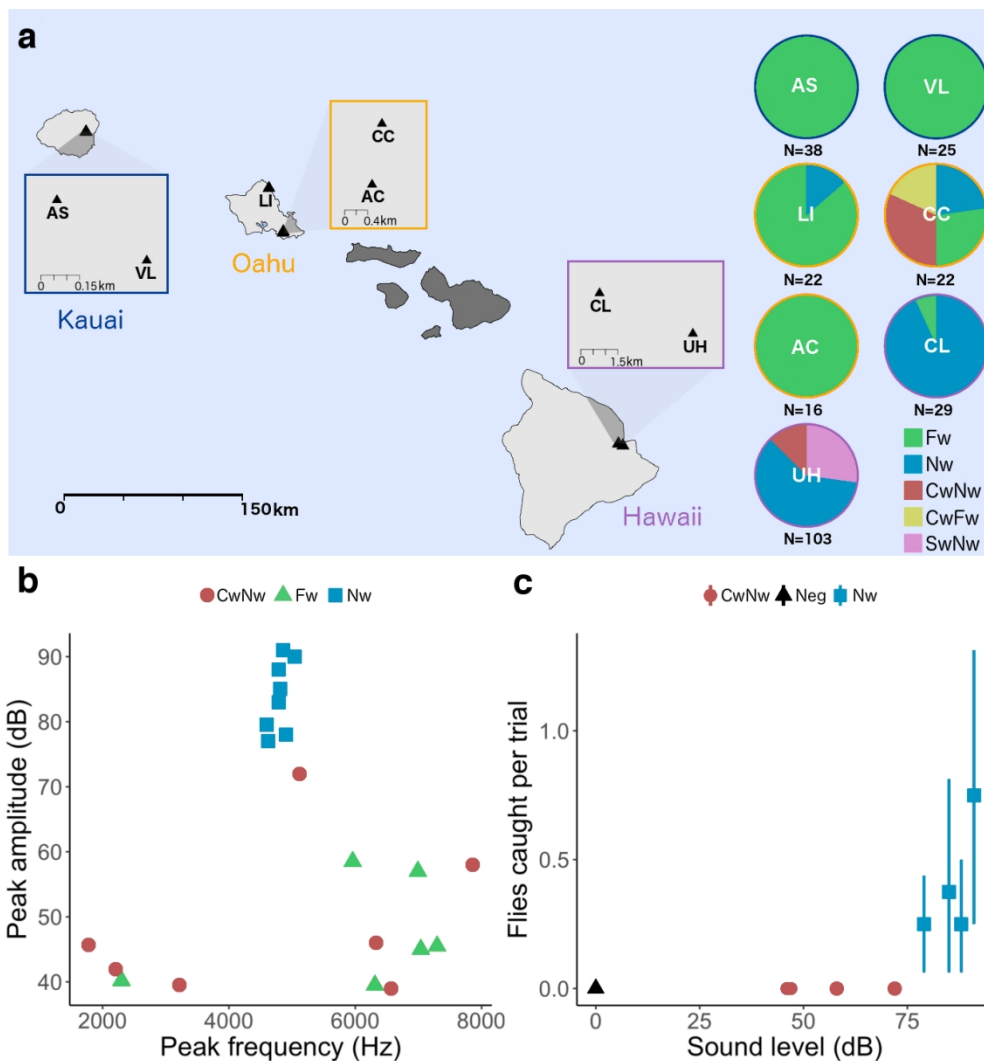


Figure 2. (A) Distributions of parasitized populations of *T. oceanicus* and proportions of males showing typical Fw and Nw morphology plus newly-identified CwNw, CwFw and SwNw phenotypes from 2018 surveys. Two-letter codes correspond to site IDs. (B) Differences in calling song properties for Nw, Fw and CwNw males recorded using a Sennheiser ME66 microphone 5 cm from subjects in the lab. (C) Flies attracted to CwNw, negative control and Nw playbacks in the field: points illustrate means and bars are 95% non-parametric confidence intervals.