

intercepts of the fitted curves can be obtained as functions of the extreme value distribution as described in the S-Plus Guide to Statistics<sup>24</sup>.

Fecundity was calculated using our modification of the triangular fecundity function<sup>26</sup>, takes the form:

$$\ln(M_x) = M_\infty (1 - e^{-(kx+t_0)}) e^{-bx} \quad (1)$$

where  $M_x$  is the fecundity at age  $x$ , and the parameters  $M_\infty$ ,  $k$ ,  $t_0$  and  $b$  were fitted by minimizing the sums of squares.  $M_\infty$  is the potential maximum daily fecundity (log transformed).  $t_0$  is the first time period of offspring production, where each unit of time equals 30 days.  $k$  characterizes the rate of increase in fecundity at age  $x$  whereas  $b$  characterizes the rate of decrease in fecundity at age  $x$ . At early ages the term  $(1 - e^{-(kx+t_0)})$  dominates and accounts for the rise in fecundity, whereas at later ages the term  $e^{-bx}$  dominates and largely determines the rate of decline in fecundity. The age at which fecundity is greatest,  $D_{\max}$ , is given by

$$D_{\max} = \frac{1}{k} \left( \ln \left( \frac{b+k}{b} \right) + t_0 \right) \quad (2)$$

Although  $b$  is a measure of the decline in fecundity, its value is also influenced by the increasing phase of the fecundity curve. A second measure of the rate of decline that is independent of the increasing phase is a linear regression of fecundity on age for ages beyond  $D_{\max}$ ; we refer to the slope of this regression as  $b_{lin}$  (Supplementary Information). We used  $D_{\max}$  for statistical comparisons among treatment groups of the age at maximum fecundity and  $b$  and  $b_{lin}$  for the rate of decline in fecundity with age. Both  $b$  and  $b_{lin}$  differed significantly among drainages;  $b_{lin}$  also differed among food levels. Both categories of variables can serve as indices of senescence. Standard errors for the estimates were obtained by the delete-one jackknife<sup>31</sup> (Supplementary Information). To remove heteroscedasticity we used the rank-transformed values of  $b$ . The pseudovalues from the jackknife procedure were used to test for variation due to drainage, food ration, predation type and all interactions.

Performance was evaluated for 9–13 fish from each population at both ages. Food effects were not significant after performance was size corrected and was not included in the analysis. Fish were placed in a glass tank with a 1 cm<sup>2</sup> reference grid on the bottom. This focal tank was in turn placed within a larger glass tank that helped regulate water temperature. Fast-starts were elicited then filmed at 500 frames per sec (Redlake Motionscope camera) and recorded to VHS videocassette tape. Recorded sequences were converted from analogue (VHS) to digital format and saved as AVI files. The dorsal midlines of the first three tail beat cycles were digitized using a modification of the public domain NIH Image program (available at <http://rsb.info.nih.gov/nih-image/>) for the Apple Macintosh. We fit a cubic spline function to each digitized midline within a sequence and used this function to find the coordinates of an unmeasured landmark at 0.35 total length from the tip of the head, which we used as a proxy for the centre of mass. We used a MSE quintic spline algorithm to smooth the displacement versus time data and to estimate instantaneous velocities and accelerations throughout a digitized sequence. From the fitted functions, we computed maximum accelerations occurring within the first 22 ms. The analysis was a repeated measures Anova with predators, drainage and time as main effects. Drainage and interactions between drainage and other variables were not significant. Maximum acceleration was the dependent variable. See ref. 32 for additional details.

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## Population density drives the local evolution of a threshold dimorphism

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**Evolution can favour more than one reproductive tactic among conspecifics of the same sex**<sup>1,2</sup>. Under the conditional evolutionarily stable strategy, individuals adopt the tactic that generates the highest fitness return for their status: large males guard females, whereas small males sneak copulations<sup>3,4</sup>. Tactics change at the status at which fitness benefits switch from favouring one tactic to favouring the alternative<sup>1,5</sup>. This ‘switchpoint’ is expressed in many species as a threshold between divergent morphologies<sup>3</sup>. Environmental and demographic parameters that influence the relative fitness of male tactics are predicted to determine a population’s switchpoint<sup>1,5</sup> and consequently whether the population is monomorphic or dimorphic. Here we show threshold evolution in the forceps dimorphism of the European earwig *Forficula auricularia* and document the transition from completely monomorphic to classical male-dimorphic populations over a distance of only 40 km. Because the superior fighting ability of the dominant morph<sup>6</sup> will be more frequently rewarded at high encounter rates, population density is likely to be a key determinant of the relative fitness of the

alternative tactics, and consequently the threshold. We show that, as predicted, population density correlates strongly with the shift in threshold, and that this factor drives the local evolution of the male dimorphism in these island populations. Our data provide evidence for the origin of phenotypic diversity within populations<sup>7–9</sup>, through the evolution of a switchpoint in a conditional strategy that has responded to local population density.

The morphological adaptations of males using alternative tactics under the conditional evolutionarily stable strategy (ESS) represent an extreme case of phenotypic plasticity<sup>9</sup>. In the European earwig *F. auricularia*, there has been a longstanding interest in this plasticity, in which the alternative male tactics are characterized by a dimorphism in male forceps length<sup>10–12</sup> (Fig. 1a, b). The dimorphism is characterized by a large-bodied ‘macrolablic’ morph with long forceps, and a small ‘brachylablic’ morph with short forceps. Male *F. auricularia* of both morphs use their forceps in courtship<sup>13</sup> and fighting<sup>6,14</sup> in which macrolablic males are

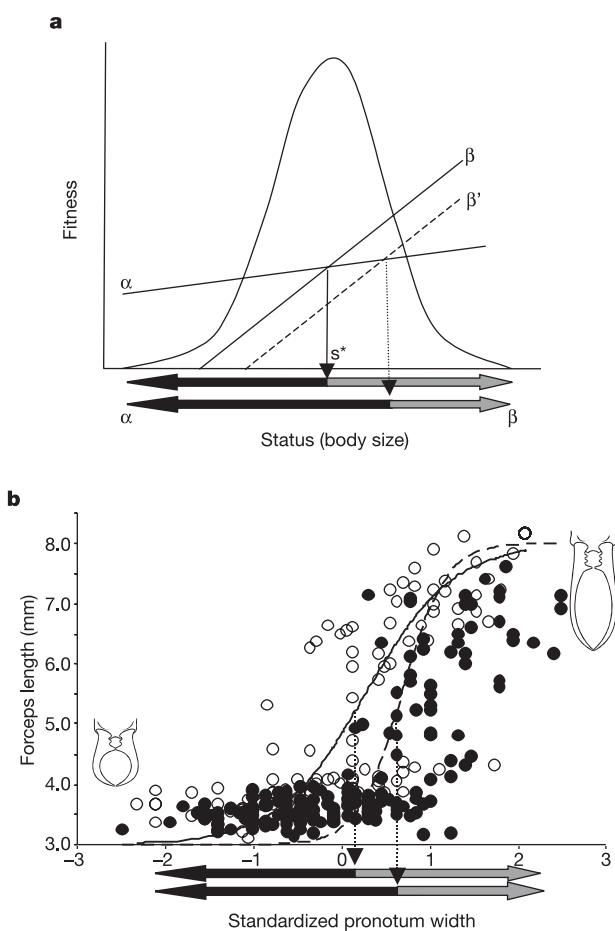
competitively superior<sup>6</sup>. Data that quantify sexual selection in the field show that macrolablic males are more likely to be found guarding a female than are brachylablic males (J.L.T., unpublished data). This is consistent with behavioural roles in which macrolablic males guard females and brachylablic males are forced to sneak copulations. The male dimorphism led early researchers to consider that the macrolablic males were a different species, *Forficula forcipata*<sup>11</sup>; however, ‘common-garden’ rearing experiments show that, within populations, the dimorphism is largely dependent on the nutrition of the developing nymph<sup>12</sup>. Subtle between-population variation in morphological threshold has been documented in a number of species reared under common-garden conditions, including Farne island populations of *F. auricularia*<sup>12</sup>, the mite *Sarcophaga berlesei*<sup>15</sup> and the dung beetle *Onthophagus taurus*<sup>16</sup>. These studies suggest that differences between populations represent the genetic divergence of the ESS switchpoints between populations. Artificial selection experiments support the notion that thresholds can harbour large amounts of genetic variation<sup>17–19</sup>, fuelling population divergence.

British island populations of *F. auricularia* have a significantly higher proportion of the macrolablic male morph than do mainland populations (Fig. 2a). This phenomenon is equally true in the northeast of the British Isles as in the southwest, suggesting that the variation between islands and the mainland is not simply due to gross variation in climate or to milder microclimatic conditions on islands (air temperature at Boulmer in Northumberland is on average 1.5 °C cooler than in St Mawgan in Cornwall; Met office data). To understand why island populations of earwigs are male-dimorphic we concentrated on population variation among North Sea islands in the Firth of Forth and the Farne Islands. This minimized differences in the climate, geological history, habitat and the ecology and diversity of interacting species. Only these islands are considered in further analyses. Earwig populations in the Firth of Forth varied considerably in the observed ratio of macrolablic to brachylablic males, ranging from 0–20% and in the Farnes group from 8–45% (Fig. 2).

Variation in morph frequency is due to a change in position of the dimorphic threshold relative to the population mean body size. Hence, morph ratio variation can either be due to threshold evolution independent of mean body size, or to changes in mean body size around a static threshold, or both. The former (shown in Fig. 1a) is consistent with ESS theory under which the threshold is determined by population-specific variation in fitness functions<sup>1</sup>; the latter is likely when a single population is reared across an environmental gradient<sup>12</sup>. To determine whether populations varied significantly in the position of the body size threshold, we categorized males as macrolablic or brachylablic (see Methods), then performed a logistic regression with morph as the dependent ‘variable’, pronotum width (a measure of body size) as a covariate and island as a factor (including all island populations with at least one macrolablic male). The whole model was significant ( $\chi^2_{21} = 1,456$ ,  $P < 0.001$ ), as was the effect of pronotum width, indicating, as expected, that larger individuals are more likely to be macrolablic ( $\chi^2_1 = 1,134$ ,  $P < 0.001$ ); the population term was also significant ( $\chi^2_{20} = 154$ ,  $P < 0.001$ ), demonstrating that populations differ in the absolute position of the morphological threshold (Fig. 3a, b).

Further evidence that the position of the threshold is not fixed comes from the positive correlation between a population’s mean pronotum width and the absolute position of the morphological threshold in the population ( $r_s = 0.77$ ,  $n = 21$ ,  $P = 0.001$ ); the ESS switchpoint apparently having evolved to some extent in parallel with increasing body size.

A logistic regression model, with pronotum width standardized within each population to have a mean of zero and a standard deviation of one, revealed that populations also differed in the position of the threshold relative to the population mean (population:  $\chi^2_{20} = 508$ ,  $P < 0.001$ ). Hence, not only does the absolute



**Figure 1** The status-dependent ESS and threshold variation in *F. auricularia*. **a**, The conditional ESS model with status-dependent alternative tactics<sup>1</sup>. Fitness functions of the tactics  $\alpha$  and  $\beta$  are status-dependent. Individuals adopt the tactic from which they derive the highest fitness; hence the proportion of males of each morph in the population is determined by the position of the intersection of the fitness functions: the ESS switchpoint  $s^*$ . Environmental and demographic variables that affect the fitness functions (for example, a decline in density shifting  $\beta$  to  $\beta'$ ) generate population variation in  $s^*$  and hence between-population variation in morph ratio<sup>1</sup> (shaded arrows). **b**, The nonlinear relationship between male body size and forceps length that characterizes male dimorphic populations of *F. auricularia*. Inset are brachylablic and macrolablic forceps. Two populations are shown: Bass Rock (open circles) and Knoxes Reef (closed circles). Cubic splines<sup>27</sup> summarize the male dimorphism in each population. The ESS  $s^*$  is reflected by the morphological threshold between the morphs. Shifts in ESS  $s^*$  due to changes in the fitness functions of the morphs (for example, **a**) are responsible for shifts in the morphological threshold between populations.

body size threshold show variation between populations, but so too does the relative position of the threshold: the parameter that determines male morph ratio. Population variation in the morphological threshold and body size has occurred on an extremely small scale. For example, the islands of East Wideopen (EWO) and Knoxes Reef (KR) in the inner group of Farne islands are separated by just 400 m of sea, and are both connected by causeways to the same island at low tide. Nonetheless, populations of earwigs on these adjacent islands differ significantly in both size (pronotum width mean  $\pm$  s.e.m: EWO =  $2.05 \pm 0.01$ , KR =  $1.88 \pm 0.01$ ;  $t_{384} = 13.5$ ,  $P < 0.001$ ) and the absolute and relative position of the threshold (final model,  $\chi^2_2 = 209$ ,  $P < 0.001$ ; pronotum width,  $\chi^2_1 = 196$ ,  $P < 0.001$ ; island,  $\chi^2_1 = 26.7$ ,  $P < 0.001$ ; using standardized pronotum width, island =  $\chi^2_1 = 19.1$ ,  $P < 0.001$  Fig. 3c).

Theoretically, the increase in frequency of macrolablic males on island populations occurs because the fitness function of macrolablic males is elevated relative to that of brachylablic males<sup>1,5</sup>. Male earwigs guard females in chambers under stones and driftwood; following oviposition, the female will not re-mate and expels the male. In *F. auricularia*, macrolablic males have an advantage over brachylablic males when competing for females (unpublished data)<sup>6</sup>. Nevertheless, adaptations for mate guarding, such as elongate forceps, are likely to be advantageous only where challenges occur regularly. The intensity of sexual selection is predicted to vary with certain demographic and ecological parameters, such as density, the spatial distribution of resources and the operational sex ratio (OSR)<sup>20,21</sup>. In *F. auricularia*, the probability of being challenged by an unpaired male, or conversely of successfully usurping a guarding male, depends on the probability of encountering another burrow. Across the very similar island habitats studied here, the predominant factor determining encounter rate is likely to be population density. Where earwigs occur at high densities, challenges over guarded females are likely to be frequent, elevating the fitness of males with adaptations for fighting for or defending females. Extremely high population densities of earwigs are a conspicuous

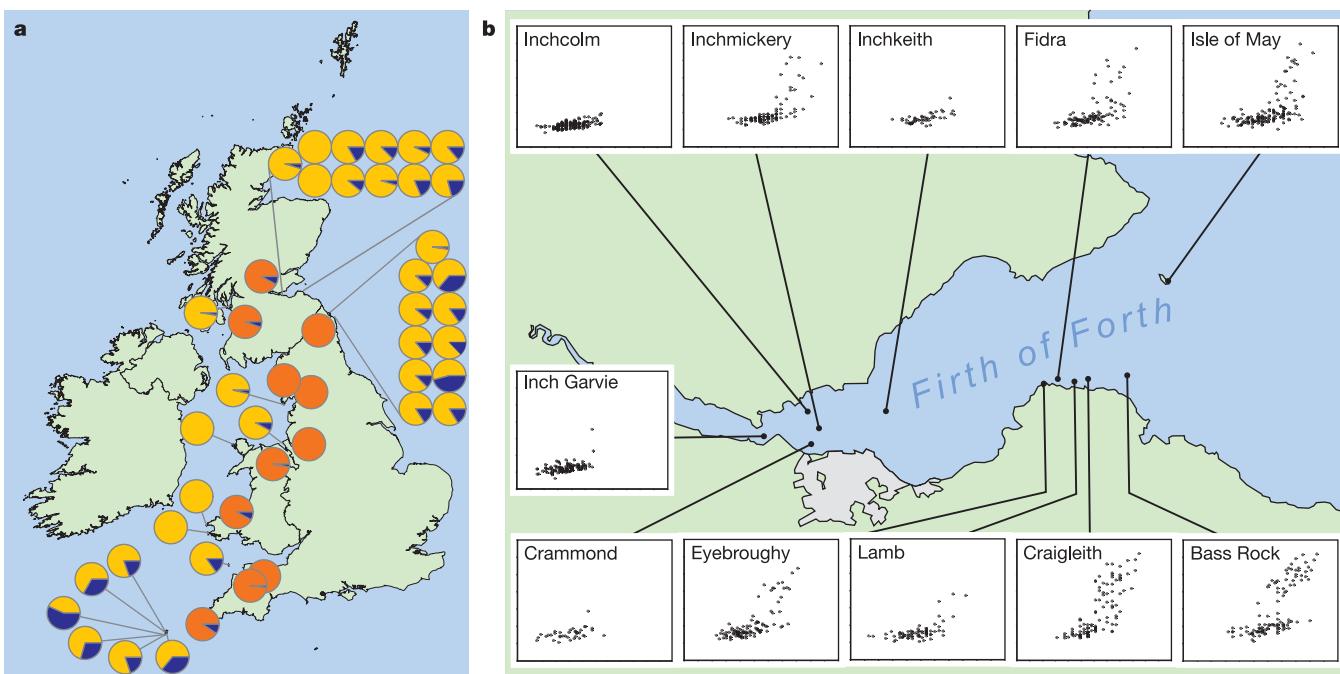
feature of the ecology of the islands of the Firth of Forth and Farnes. Across the North Sea island populations, we found that there was a significant relationship between the overall density of the earwigs and the proportion of macrolablic males in the population ( $r_{s22} = 0.66$ ,  $P = 0.001$ , Fig. 4).

OSR is an important factor determining the intensity of sexual selection<sup>20</sup>. Although the sex ratio did become more male biased as population densities increased across islands, there was no significant relationship with male morph ratio (see Supplementary Information).

Threshold evolution in earwigs on these North Sea islands has taken place in similar climatic conditions and similar habitats. The extraordinarily high population densities that seem to be driving threshold evolution may arise due to the escape from parasitic species or competitors present on the mainland. This hypothesis predicts increasingly high densities of earwigs as islands become more difficult for other species to colonize. There was a significant increase in earwig density with increasing distance of the island from the mainland ( $r_{s22} = 0.616$ ,  $P = 0.002$ ), but no increase in density with decreasing island size ( $r_{s22} = -0.05$ ,  $P = 0.836$ ). Despite the increase in density with distance to the mainland, there was no increase in the proportion of macrolablic males with distance to the mainland ( $r_{s22} = 0.36$ ,  $P = 0.101$ ).

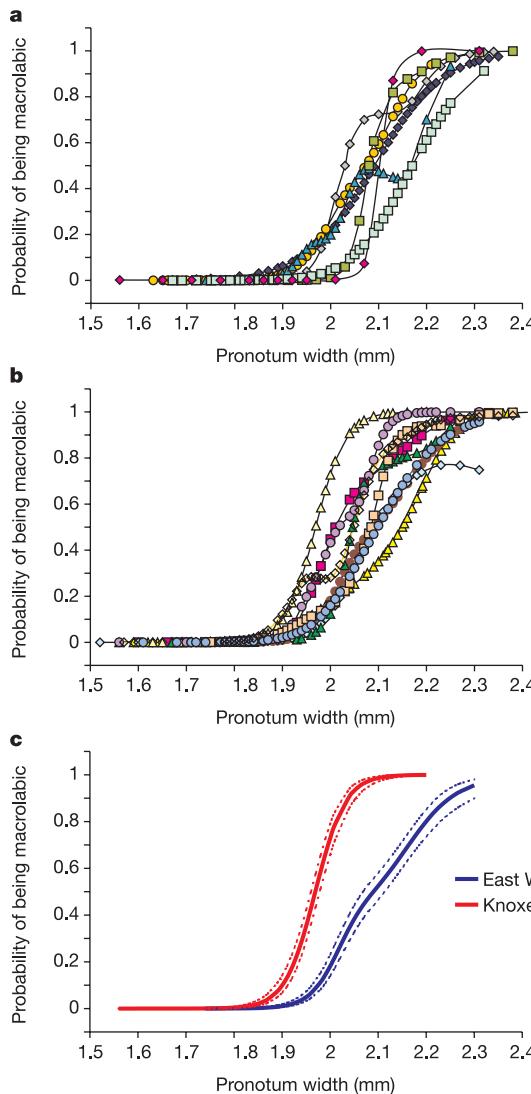
The input of organic material on which earwigs feed may also be an important determinant of population density on small islands with sparse vegetation. Earwigs eat both guano and the carcasses of dead seabirds. Nevertheless, the biomass of ground-nesting birds per square metre on each island did not correlate with earwig density ( $r_{s21} = 0.02$ ,  $P = 0.915$ ).

We have shown that threshold evolution can occur on an extremely small geographic scale in a pattern consistent with local variation in demographic parameters that are theoretically important to variation in the intensity of sexual selection<sup>21</sup>. Male-dimorphic threshold traits have been shown to respond readily to selection<sup>17,19</sup>, and although population variation consistent with

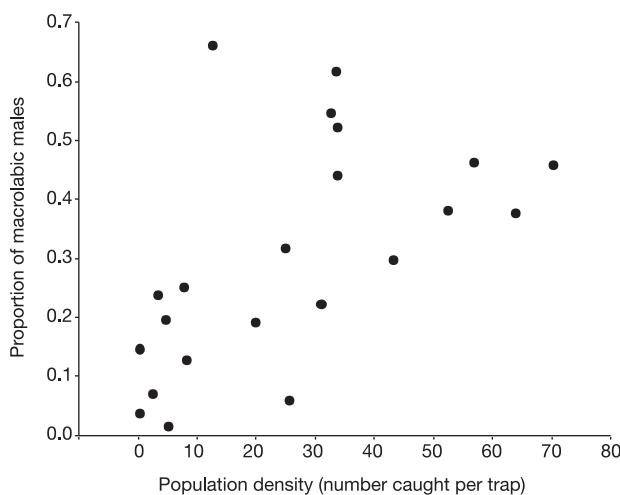


**Figure 2** Morph ratio variation among populations of *F. auricularia*. **a**, Map of Britain and Ireland with pie charts showing the proportion of male morphs in island and mainland populations of the European earwig *F. auricularia*. Macrolablic males represent a greater proportion of males in island populations than mainland populations ( $t$ -test on arcsine square-root transformed proportion macrolablic,  $t_{44} = 3.17$ ,  $P = 0.003$ ). Macrolablic

males (blue); brachylablic males in mainland populations (orange) and in-island populations (yellow). **b**, Variation between island populations in the Firth of Forth. Inset scatter plots show the relationship between forceps length (y axis, all graphs 2.0–9.0 mm) and pronotum width (x axis, all graphs 1.4–2.5 mm).



**Figure 3** Variation in threshold among island populations of *F. auricularia*. **a, b**, Cubic splines summarizing the threshold variation in the reaction norm of forceps length on pronotum width for populations of *F. auricularia* in the Firth of Forth (**a**) and the Farne Islands (**b**). **c**, Population variation in threshold on a very small geographic scale: Knoxes Reef and East Wideopen are islands in the Farnes group and are about 400 m apart. Dashed lines are 95% confidence intervals for the splines.



**Figure 4** The relationship between density, estimated as the average number of earwigs caught in standard traps, and the proportion of macrolabic males in a population, for populations of *F. auricularia* from islands in the Firth of Forth and Farnes.

change in density has been proposed for dung beetles, the results were inconclusive<sup>22</sup>. Unlike previous studies, we have demonstrated that threshold evolution can tailor populations to fine-scale variation in demography. This is expected in threshold traits that reflect status-dependent alternative reproductive tactics, because any differences in the slopes or elevations of the fitness functions of alternative tactics affect the position of the ESS switchpoint (Fig. 1), thereby making it particularly sensitive to changes in parameters that alter relative fitness (see ref. 21 for an alternative perspective on the status-dependent ESS model). We are currently examining the extent to which gene flow between islands and with the mainland influences threshold variation among these populations.

We have documented a micro-evolutionary transition within a single species, over less than 40 km, from populations in which the ESS is for a single tactic expressed by all individuals, through to a classic conditional strategy in which alternative tactics are played in a status-dependent manner. These populations provide us with snap-shots in the evolution of a male dimorphism and support the game theoretic premise that ESSs are population specific<sup>1,2</sup>. □

## Methods

### Collections

Earwig samples from the west coast and west-coast islands of Britain were collected from beneath driftwood and stones, or from the hollow stems of the hogweed *Heracleum sphondylium* (Umbelliferae). Collections were made between August and October 2002 (average sample size,  $72 \pm 9$ ; range, 13–148). Trap sampling of the Farne Islands was carried out in August 2001 and of the Firth of Forth in September 2002 (average sample size,  $121 \pm 11$ ; range, 37–227). Samples were stored at  $-20^{\circ}\text{C}$  before measurement.

### Measurements

Male earwigs from 22 focal islands in the Firth of Forth (Inchcolm,  $n = 145$ ; Inch Garvie,  $n = 100$ ; Crammond,  $n = 37$ ; Inchmickery,  $n = 92$ ; Inchkeith,  $n = 145$ ; Eyebroughy,  $n = 100$ ; Lamb,  $n = 71$ ; Fidra,  $n = 100$ ; Craileath,  $n = 100$ ; Bass Rock,  $n = 105$ ; Isle of May,  $n = 138$ ) and the Farne Islands (Lindisfarne,  $n = 73$ ; Inner Farne,  $n = 82$ ; Knoxes Reef,  $n = 192$ ; West Wideopen,  $n = 227$ ; East Wideopen,  $n = 194$ ; Staple,  $n = 224$ ; Brownsman,  $n = 176$ ; South Wamses,  $n = 135$ ; North Wamses,  $n = 123$ ; Big Harcar,  $n = 57$  and Longstone End,  $n = 58$ ) were measured under a Leica MZ5 binocular microscope with an eyepiece graticule, or using Scion Image (NIH) software. Male pronotum width and right forceps length were measured. Male earwigs from west coast mainland and island populations were used only in the analysis of geographic variation in morph ratio. This analysis does not require precise knowledge of the morphological switch point and morphs were assigned by eye.

### Switchpoint/threshold estimation

We have used the term 'threshold' to describe the morphological transition between morphs<sup>23</sup>; the threshold estimates the position of the ESS 'switchpoint'<sup>1</sup>. Thresholds were estimated using the method described in ref. 24 (hereafter referred to as the E&G method). We used a program written for this procedure in Splus 2000, in which 100 possible thresholds could be tested per population (K. Wilson). We chose the threshold at which the  $r^2$  and the significance of the  $\beta_3$  inflection were maximized. Using the E&G method, the proportion of the population that lie each side of the morphological threshold was calculated as the proportion of a normal curve<sup>25</sup>. This estimate of morph ratio was used to compare populations independent of among population variation in body size. Thus the E&G method was used to estimate the morphological threshold and population morph ratio. In addition, Kotiaho and Tomkins' (K&T) modification of the E&G method was used to assign earwigs to different morphs on the basis of their forceps length<sup>26</sup>. These data were used in the logistic regression analyses of population variation in threshold. The K&T method was also used to summarize the relationship between forceps length and pronotum width using cubic splines (Fig. 3a–c). Output from the cubic spline program glmsWIN10<sup>27</sup> was used to interpolate the body size at which males were macrolabic with a 50% probability; this K&T threshold correlated strongly with the E&G threshold ( $r_{s22} = 0.88$ ,  $P < 0.001$ ).

### Density

Earwig density was estimated from trap catches. Earwigs aggregate in crevices during the day and traps were designed to exploit this behaviour. Traps were 150-ml cylindrical plastic vials containing a roll of standard corrugated cardboard (see Supplementary Information for further details). The average number of traps per island was  $22 \pm 2$ . After 3 weeks we returned to the islands and collected the earwigs residing in the traps. Analysis of variance (ANOVA) with population as a random effect revealed that there was significant variance between islands in trap catches ( $F_{21,502} = 9.57$ ,  $P = 0.001$ ). Density data per island were left-skewed and on the verge of non-normality (Shapiro-Wilk = 0.91, d.f. = 21,  $P = 0.05$ ) and because log transformation did not normalize the data nonparametric statistics were used.

### Biomass of nesting birds

The number of pairs of the predominant species of ground-nesting birds nesting on each island of interest was calculated for the Farne Islands using the records of the National

Trust (1924–2003), and for the Firth of Forth using data from the Seabird Monitoring Program (1969–2003). The biomass of nesting birds per island was calculated using body weight data<sup>28</sup> (see Supplementary Information for exact details and species).

### Island area and habitat

Island area was calculated from 1:25,000 maps obtained from the Ordnance Survey website and saved as JPEG files. Area within the mean high-water contour was measured using Scion Image. The islands are small; 17 out of the 22 have an area above the mean high-tide level, equivalent to a circle with a diameter of less than 270 m. This area will be an overestimate of the land habitable to earwigs because of spray, spring tides and storms. The islands are rock with a cap of shallow soil or peat, which is restricted to rock fissures on Longstone End, Eyebroughy and Big Harcar. Knoxes Reef is a shingle bank that has built up behind a reef of rock. The vegetation on all of the islands is dominated by grasses and annual herbs. Only Crammond and Lindisfarne support trees.

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## Non-mitochondrial complex I proteins in a hydrogenosomal oxidoreductase complex

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*Trichomonas vaginalis* is a unicellular microaerophilic eukaryote that lacks mitochondria yet contains an alternative organelle, the hydrogenosome, involved in pyruvate metabolism. Pathways between the two organelles differ substantially: in hydrogenosomes, pyruvate oxidation is catalysed by pyruvate-ferredoxin oxidoreductase (PFOR), with electrons donated to an [Fe]-hydrogenase which produces hydrogen. ATP is generated exclusively by substrate-level phosphorylation in hydrogenosomes, as opposed to oxidative phosphorylation in mitochondria<sup>1</sup>. PFOR and hydrogenase are found in eubacteria and amitochondriate eukaryotes, but not in typical mitochondria<sup>2–4</sup>. Analyses of mitochondrial genomes indicate that mitochondria have a single endosymbiotic origin from an  $\alpha$ -proteobacterial-type progenitor<sup>5</sup>. The absence of a genome in trichomonad hydrogenosomes<sup>6</sup> precludes such comparisons, leaving the endosymbiotic history of this organelle unclear<sup>7</sup>. Although phylogenetic reconstructions of a few proteins indicate that trichomonad hydrogenosomes share a common origin with mitochondria<sup>8–11</sup>, others do not<sup>2–4,7</sup>. Here we describe a novel NADH dehydrogenase module of respiratory complex I that is coupled to the central hydrogenosomal fermentative pathway to form a hydrogenosomal oxidoreductase complex that seems to function independently of quinones. Phylogenetic analyses of hydrogenosomal complex I-like proteins Ndh51 and Ndh24 reveal that neither has a common origin with mitochondrial homologues. These studies argue against a vertical origin of trichomonad hydrogenosomes from the proto-mitochondrial endosymbiont.

To establish the ancestry of *T. vaginalis* hydrogenosomes and investigate whether hydrogenosomes derive from the proto-mitochondrial endosymbiont, we have taken a proteomics approach. Two-dimensional polyacrylamide gel electrophoretic analyses of soluble hydrogenosomal fractions have revealed a 48-kDa protein, Ndh51 (NADH dehydrogenase, 51 kDa), which is 57% identical to the 51-kDa subunit of mitochondrial respiratory complex I and to the NuoF (NADH:ubiquinone oxidoreductase, chain F) subunit of proteobacterial complex I. Complex I catalyses electron transfer from NADH to quinone and generally translocates protons across bacterial or mitochondrial membranes, creating an electromotive force that drives ATP production<sup>12</sup>. Proteobacterial complex I contains 14 subunits, NuoA to NuoN, organized in three functional domains: an NADH dehydrogenase, a quinone-binding module, and a proton translocation module<sup>12</sup> (Fig. 1a). In addition to these core subunits, mitochondrial complex I also comprises up to 32 subunits of non-endosymbiotic origin<sup>12,13</sup>. NuoF, NuoE and NuoG form the NADH dehydrogenase module bearing