Evolutionary fine-tuning of background-matching camouflage among geographic populations in the sandy beach tiger beetle

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Abstract

Background-matching camouflage is a widespread adaptation in animals; however, few studies have thoroughly examined its evolutionary process and consequences. The tiger beetle *Chaetodera laetescripta* exhibits pronounced variation in elytral colour pattern among sandy habitats of different colour in the Japanese Archipelago. In this study, we performed digital image analysis with avian vision modelling to demonstrate that elytral luminance, which is attributed to proportions of elytral colour components, is fine-tuned to match local backgrounds. Field predation experiments with model beetles showed that better luminance matching resulted in a lower attack rate and corresponding lower mortality. Using restriction site-associated DNA (RAD) sequence data, we analysed the dispersal and evolution of colour pattern across geographic locations. We found that sand colour matching occurred irrespective of genetic and geographic distances between populations, suggesting that locally adapted colour patterns evolved after the colonisation of these habitats. Given that beetle elytral colour patterns presumably have a quantitative genetic basis, our findings demonstrate that fine-tuning of background-matching camouflage to local habitat conditions can be attained through selection by visual predators, as predicted by the earliest proponent of natural selection.

Keywords: camouflage, character evolution, colouration, local adaptation
1. Introduction

Organismal appearance traits such as body colour pattern are strikingly diverse and have been widely studied as evidence for evolution by natural selection and biodiversity [1–3]. Anti-predator adaptation is among the most important evolutionary processes, and is responsible for a myriad of putative camouflage patterns to avoid detection by visually oriented predators [2–6]. A typical form of camouflage comprises cryptic colouration, often referred to as background matching, which conceals individuals through similarities in colouration with their natural background [4,5,7]. A fundamental prediction for this type of camouflage is the evolution of a single optimal body colour that is fine-tuned to match the background colouration, with respect to predator vision, thereby maximising the efficacy of concealment [7–10].

Previous studies have provided empirical and comparative evidence to support the hypothesis that selection via predation can lead to background matching in animal colouration (e.g., [11,12]). However, few studies have quantitatively examined how closely prey colour matches the background from the perspectives of predator vision and the efficacy of predation risk reduction in natural conditions [13,14]. A fundamental criterion of camouflage theory is that closer matching of an object to its background will reduce the likelihood that it is found and attacked by predators [5]. Wild animals such as avian predators have visual systems that differ from the human system in terms of the number of receptor types, receptor sensitivity, and ability to perceive ultraviolet (UV) light [15]. Therefore, for effective camouflage, prey animals must closely resemble their background in appearance with respect to predator vision; the resemblance must directly relate to survival against predators in the wild. Recent advances in optical analysis techniques and knowledge of colour perception have allowed modelling of predator vision and consideration of how colouration might be perceived by natural predators [12,16]. Some studies have used vision models to test concealment [17–21]; however, tests for optimal camouflage using actual predators remain rare [13,14]. Therefore, the evolutionary fine-tuning of cryptic colouration to match local backgrounds has not yet
been fully demonstrated, although it is a fundamental prediction of anti-predator adaptations.

Traits with multiple functions (e.g., body colouration) can be affected by both biotic and abiotic selection factors, as well as by phylogenetic constraints [1]. Therefore, it is crucial to examine how different types of selection and phylogenetic constraints have affected the evolution of body colouration, in conjunction with natural selection for camouflage [22,23]. Geographic colour variation provides a good system to study colour matching with spatial replication and to resolve the relative importance of different processes in phenotypic evolution [24]. The study of geographic variation can also provide insight into the population genetics of phenotypic evolution [25]. Background matching can occur by two processes: evolutionary fine-tuning of body colour to match background patterns after the colonisation of a new habitat, and colonisation of a habitat with background patterns that match body colour. The relative importance of these processes can be evaluated by examining the relationships of genetic, geographic, and phenotypic distances among populations.

Tiger beetles (Coleoptera: Cicindelidae) are a species-rich group with extreme colour pattern diversity, which provide an intriguing means for investigation of colour pattern evolution [26,27]. Associations between body colouration and environment are observed even among subspecies of tiger beetles, and have long been recognised as predator-avoidance camouflage [3,28]. Previous studies have shown differences in white elytral markings and activity between cool and warm periods among subspecies of tiger beetles, suggesting that thermoregulation may be another important determinant of colour pattern [29,30]. Furthermore, the body colouration of tiger beetles is determined genetically and indicates relatedness among species, suggesting that phylogenetic constraints can affect colouration [27,31,32]. The tiger beetle Chaetodera laetescripta shows pronounced geographic variation in the black and ivory colour patterns of its elytra in the Japanese archipelago (figure 1a), but not in continental Asia [33,34]. In Japan, C. laetescripta elytral colouration resembles the local habitat colouration (figure 1b), which
varies from off-white to black due to the complicated local geology [34] (figure 1a). From
north to south, the Japanese Archipelago is characterised by a climatic gradient that plays
an important role in colour divergence among insects [35,36]. Therefore, climatic factors
such as temperature and solar radiation may have played a role in the colour pattern
divergence of *C. laetescrpta* [37]. The tiger beetle depends on sparsely vegetated sandy
habitats; adults emerge during the daytime in summer (June to September) [38] and are
therefore likely to be exposed to visually guided predators (e.g., birds and robber flies)
on the bare ground, as well as to heat stress associated with their colouration [31].

The objectives of this study were to demonstrate the occurrence of fine-tuned
background matching, examine the role of selection in camouflage optimisation in terms
of geographic colour variation, and explore the evolutionary process of geographic colour
variation in the tiger beetle *C. laetescrpta*. First, we assessed how closely the elytral
colouration of the tiger beetle resembles its background from the perspective of avian
predator vision. Next, we tested the selective advantage of colour matching in artificial
models by exposing them to natural avian predators in the wild. Third, we analysed the
genetic structures of tiger beetle populations using high-throughput sequencing
(restriction site-associated DNA sequencing) [39] to investigate the evolutionary pattern
of background matching. Finally, we performed a phylogenetic comparative analysis to
test whether variations in colour patterns were primarily determined by background
colouration, climatic factors, or phylogenetic effects.

2. Materials and Methods

(a) Field sampling

*C. laetescrpta* tiger beetles were collected from 12 sites, representing nearly the entire
range of their visual appearance and distribution in Japan (figure 1a; electronic
supplementary material, table S1). Internal tissues of the beetles were preserved in 99%
ethanol at –30°C until DNA extraction; the remaining exoskeletons were stored at –30°C
and then defrosted for the collection of photographic data. At each site, sand samples were
collected at < 2 cm from the surface where the tiger beetles were collected, and then air-
dried until they could be photographed.

(b) Digital image analysis

(i) Photographic data collection
Photographs of the tiger beetles and sand samples were taken for image analysis ($n = 2–
20$ per site; electronic supplementary material, table S1) using a Nikon D7000 camera
that had previously been converted for full-spectrum sensitivity by removal of the internal
UV filter. A Nikkor 105-mm lens attached to a Baader UV/IR Cut Filter (transmitting
wavelength, 400–700 nm) was used for photographs in the human visible spectrum; the
Nikkor 105-mm lens was attached to a Baader UV Pass Filter (300–400 nm) for UV
images. Illumination was provided by an MT70D EYE Colour Arc lamp (Iwasaki), from
which the UV blocking filter had been removed to allow a full wavelength spectrum. A
cylindrical sheet of natural white polytetrafluoroethylene (PTFE) was placed around the
sample to diffuse the light evenly. All samples were photographed from a standardised
distance and angle with a scale bar and a 99% Spectralon reflectance standard (Labsphere)
to normalise images. All images were recorded in RAW format with a fixed aperture
($f/10$) and ISO sensitivity (400); exposure time was automatically selected for the correct
exposure.

(ii) Predator vision modelling and camouflage assessment
Avian visual modelling and digital image analysis were conducted using customised plug-
ins [17] in ImageJ software [40] to assess the efficacy of tiger beetle background-
matching camouflage against predators. Multispectral image stacks were first obtained
from sets of human visible and UV photographic data of each sample to prepare for image
analysis. The position of the reflectance standard was selected manually for each photo
for normalisation. As regions of interest, polygons of whole elytra were manually selected
for beetles and fixed-diameter circles (around 1000 pixels, equivalent to 15 mm) were
manually selected for sand samples, using ImageJ selection tools.

A cone-mapping model was generated to convert calibrated images from camera photoreceptor values to predator cone-catch values by means of a polynomial mapping technique [17,41] under MT70D lighting conditions in a wavelength band of 300–700 nm. Cone sensitivity values for the blue tit (Cyanistes caeruleus) [42] were selected because small birds are the most important predators of tiger beetles [43] and because the blue tit’s visual system is considered representative of those of many UV-sensitive birds [13,42,44]. In addition to birds, robber flies and lizards can also be important predators of tiger beetles [43,45]; however, they are unlikely to affect the elytral colouration of C. laetescopra because robber flies attack beetles in the air during flight [43,45] and there are no or few lizards in the habitats of this tiger beetle species in Japan.

Luminance (lightness of colour) perceived by avian predators in the selected region of each elytra and sand image was estimated using the cone-mapping model. Correlations between elytra and sand mean luminance values for each site were examined using Pearson correlation tests in R ver. 3.6.2 software [46]. Just noticeable differences (JNDs) were used, in accordance with the widely used Vorobyev–Oshiro receptor noise discrimination model [47], to evaluate discrimination between elytral and sand luminances. Each JND value was calculated from the log difference in double cone catches of two samples divided by the Weber fraction [48], which was defined as 0.05 in this study because it represents the most abundant cone type [47]. JND < 1.00 indicates that two objects cannot be distinguished even under optimal viewing conditions, while JND < 3.00 indicates that two objects cannot be distinguished under inadequate lighting conditions; JND > 3.00 indicates increasing contrast and greater differences in distinguishability [48].

(iv) Calculation of pattern coverage

The proportion of white elytral area on tiger beetles was measured using the Natsumushi image measurement software [49] to assess the relationship between luminance and colour pattern. Visible images in RAW format were converted to JPEG format using
Adobe Photoshop CC software. For each image, the whole elytra area was manually selected as the region of interest using a polygon selection tool in the Natsumushi software; images were then binarised using a global threshold based on Otsu’s discriminate analysis [50]. The proportion of white area was calculated as the ratio of pixels with higher lightness than the threshold value. Pearson’s product moment correlation was used to test correlations between luminance and white elytra areas using R ver. 3.6.2 software [46].

(d) Field predation experiments

(i) Construction of artificial tiger beetles

Field predation experiments using paper tiger beetle models were conducted to test for the selective advantage of background matching in luminance. A well-established method for field predation experiments was followed, in which a piece of printed paper and an edible mealworm were used [8,51,52]. Two representative habitats showing a large difference in beetle elytral colour pattern and sand colour were selected as experimental sites (Site J: light background, Site F: dark background; figure 1a). Light (60% of the elytral area was white) and dark models (10%) were constructed that were 15 mm in length, corresponding to the adult body length of the tiger beetle [53]. The colour patterns were printed using a Canon TS 9030 inkjet printer on pieces of Whatman filter paper (no. 1001–240), which has neutral reflective properties and is often used in predation research [13,54]. The image analysis described above was conducted to evaluate differences in luminance between the paper model and background sand for avian viewing. Photographic data were obtained for both models (n = 12 per type) and sand samples collected from the experiment sites (n = 12); JND values were calculated between models and sand samples. The paper model was attached to a black straw (15 mm in length, 3.5 mm in diameter) using double-sided tape; a dead mealworm (Tenebrio molitor larva; 10–15 mm in length; frozen overnight and then thawed) was pinned into the straw with its tail protruding approximately 5 mm from the end.
Experimental trials were conducted at both sites (Site J and F) from August to September 2018. At each site, three transects were simultaneously set at a distance of > 100 m apart. Twenty-five models of each type were alternately placed at distances of > 2 m apart on each transect. A model horizontal was fixed to the ground surface at a height of approximately 5 mm by using a thin wire stuck into the ground. Model presentation trials began at 4:00 AM and 4:00 PM (Japan Standard Time) and were completed within 30 min; the survivorship of the models was examined 3 h later. This procedure was repeated for 5 consecutive days, except on rainy days. During the experiments, insect-eating birds including sparrows (Passer montanus), meadow buntings (Emberiza cioides), and crows (Corvus corone) were commonly observed to walk and search for prey on the ground at both sites (electronic supplementary material, table S2); the models were attacked upon disappearance of the mealworms. The final sample size was \( n = 2983 \) (Site J: 747 of each model; Site F: 743 and 746 light and dark models, respectively) after exclusion of data for 19 models (Site J: 3 light, 3 dark; Site F: 7 light, 4 dark) that had been lost or swarmed by non-avian foragers such as ants.

**Statistical analyses**

Generalised linear mixed model analysis with binomial error distribution was conducted to examine the effect of background resemblance on the model attack rate. Whether models were attacked or not (1 or 0) was used as the independent variable; model colour (light or dark) was used as the explanatory variable. Start time (4:00 AM or 4:00 PM), transect number (1–3), and days (1–5) were included as random effects. These analyses were conducted in R ver. 3.6.2 [46] using the ‘glmer’ function in the lme4 package [55] and the ImerTest package [56] for \( p \) value calculation.

**Phylogenetic analysis with RAD sequencing**

*Rad sequencing and phylogenetic tree estimation*

Four to five individuals in each population from a total of 13 sites were used, including...
samples from South Korea as the outgroup (figure 1a; electronic supplementary material, table S1). Total genomic DNA was extracted from thorax muscles or testicles using a DNeasy Blood & Tissue Kit (Qiagen). For each sample, approximately 25 ng/μL (10–30 ng/μL) of genomic DNA was digested using the restriction enzyme PstI in NEB Buffer4, then ligated with a P1 adaptor and a unique five-base sequence. Library construction and single-end 101-bp sequencing were performed using a HiSeq 2500 sequencer (Illumina) at Hokkaido System Science, Sapporo, Japan.

The RAD sequence data were processed using the ipyrad ver. 0.9.31 software [57]. Sequences were identified as homologous and clustered when they had greater than 90% similarity. Loci with sequences that were shared by more than 55 samples were retained in the final dataset. The phylogenetic tree was estimated using the maximum likelihood (ML) method using the RAxML ver. 8.2.12 software [58]. A general time-reversible model with gamma probability distribution was used as the evolutionary model; node support values were calculated by rapid bootstrapping analysis with 100 replications [59].

(ii) Mantel test and phylogenetic comparative analysis

The relationships among inter-population differences in body colour, genetic distance, and geographical distance were investigated by means of the Mantel test [60] with 1000 repetitions using the ‘Mantel’ function in the ape R package [61] in R ver. 3.6.2 [46]. The mean proportion of the white area in tiger beetles was calculated for each site in Japan (electronic supplementary material, table S1); pairwise Euclidean distances between the mean values were obtained in R. Genetic distance was calculated as pairwise FST between population samples by means of the ‘fst’ function in the GENEPOP software package [62], using files converted from ipyrad output using the PGDSpider conversion tool [63]. Geographical distance was calculated as the great circle distance (km) among sites using latitude and longitude information for each site and the ‘distm’ function in the geosphere R package [64].

The effects of background colouration and thermal environment on inter-population differences in colour pattern were also examined by using phylogenetic generalised least-
squares (PGLS) analysis based on a Brownian motion model of trait evolution [65,66]. For the PGLS analysis, one individual per site, which had the lowest proportion of missing RAD loci among individuals from the same site, was selected, and an ML phylogenetic tree was constructed using the general time-reversible model with gamma probability distribution in the RAxML software. The tree was converted into ultrametric form using the penalised likelihood method with the ‘chronos’ function in the ape R package. As climatic factors, mean temperature (°C) and mean global solar radiation (MJ m⁻²) data for the adult activity period (June–September) [38] were used; these were obtained from 1-km mesh meteorological data (Mesh Climatic Data 2000; Japan Meteorological Business Support Center; electronic supplementary material, table S1). The effects of hypothetical factors (mean sand luminance and the two climatic factors) on the mean values of white area proportion (%) were examined by the PGLS analysis using the ‘gls’ function in the nlme R package [67]. Concomitantly, the likelihoods of these models were compared using Akaike’s information criterion values.

3. Results

(a) Body colour pattern and sand colour

We collected photographic data from 193 tiger beetles and sand samples taken at 12 sites (figure 1a; electronic supplementary material, table S1 and S3). The mean luminance values of elytra and sand at each site varied geographically and were correlated with each other across populations (Pearson's product moment correlation: \( r = 0.86, P < 0.001; \) figure 2a). The average JND between tiger beetle body and sand luminance was as low as 1–5 at most sites. Thus, tiger beetles had site-specific body colouration patterns that were well matched to the background colour in terms of avian vision.

The elytral white area proportion varied from 6.1% to 74.1% (electronic supplementary material, table S3) and increased with increasing luminance (\( r = 0.96, P < 0.001; \) figure 2b). Thus, elytral luminance was determined by the extent of white colour
marking among elytra.

(b) Camouflage effect of luminance matching

JND values between the luminance of two tiger beetle models used in the field experiment were greater than 3 (mean ± standard deviation: 27.8 ± 1.64), suggesting that avian predators were able to easily distinguish the models according to luminance. In addition, the light model had a lower JND value on light sand (5.21 ± 2.19) than the dark model (34.2 ± 3.84), whereas the dark model had a higher JND value on light sand (22.6 ± 2.56) than the dark model (6.40 ± 4.02). Therefore, we presume that predators were able to easily distinguish the colour of one model, but not the other, from the sand colour.

In total, 127 models were attacked at the light sand site (Site J) and 150 at the dark sand site (Site F) throughout the experimental period (electronic supplementary material, table S4). Light models were attacked significantly less frequently than dark models at the light background site (generalised linear mixed model: \( z = -2.790, P = 0.005 \); figure 3, left), but more frequently than dark models at the dark background site (\( z = 2.521, P = 0.012 \); figure 3, right). Thus, colour patterns that better matched the background were associated with higher survival rates.

(c) Colour pattern evolution

We obtained a total of 2.6 million RAD sequences from 64 individuals. The ML tree showed monophyly of each population with > 80% bootstrap support (figure 4a). The pairwise genetic distance (\( F_{ST} \)) increased with the pairwise geographic distance among sampling sites (Mantel test: \( P = 0.009 \); figure 4b), revealing an isolation-by-distance pattern [68]. However, genetic distance was not related to the difference in the mean proportion of white area (\( P = 0.375 \); figure 4c).

The best model, with the smallest Akaike’s information criterion value, for the proportion of white elytral area was the model that included only mean sand luminance as a predictor variable, which had a positive effect on the proportion of white area (PGLS:
Climatic factors had no significant effects on the proportion of white area among models that included climatic variables (table 1).

4. Discussion

(a) Fine-tuning of body colour to local backgrounds for camouflage

In this study, we demonstrated that the elytral colour of C. laetescrpta closely matched the luminance of the local background substrate in the context of avian vision (figure 2a), suggesting local adaptation for predation avoidance. The field predation experiment showed that better-matched models were attacked less frequently (figure 3), suggesting that better-matched colouration is effective for reduction of predation risk. These results implied that predation-induced selection of elytral colour resulted in single optimal colourations that were finely tuned to each visual background, supporting the fundamental prediction of camouflage theory [5]. Our results confirmed the role of cryptic colouration in the tiger beetles, which was initially documented by Alfred Russel Wallace in the 19th century [3,28,69,70].

The proportion of white maculation area showed continuous variation among individuals and a robust positive correlation with elytral luminance (figure 2b). The maculation patterns of tiger beetles are caused by the absence of the black pigment melanin and are determined genetically [31]. Thus, maculation pattern variation likely has a polygenic basis, which allows beetle populations to evolve towards optimal luminance against a wide range of background colouration. In nature, animal camouflage with genetically determined colouration may be more widespread than the camouflage determined by morphological or physiological colour changeability, which is observed in cuttlefish, cephalopods, and crabs [71]. However, most studies of animal camouflage showing fine-tuning to local backgrounds have dealt with species exhibiting colour changeability [12,14]; the exceptions are studies of melanin-pigmented fur or skin among vertebrates [21,72]. Thus, our study is one of few that have demonstrated finely tuned
background-matching camouflage in detail in a species with genetically determined
colouration. Notably, we demonstrated that camouflage colouration is adjusted by the
dark/light colour ratio, which is a common aspect of animal appearance and can also be
related to thermoregulation and aposematism [73,74].

Body colouration patterns can also influence camouflage in various ways. The
degree of matching in colour pattern spatial characteristics (e.g., grain and geometry) to
background can also affect survival [5,7,75]. In addition, background-matching
camouflage may contain disruptive colouration, which can interfere with the image of the
animal as detected by the predator, thereby enhancing predator avoidance [76]. In tiger
beetles, the intricate boundary between high-contrast patterns (figure 1) could function as
disruptive colouration, whereby some markings create false edges or blend into the
background [70,77]. These colour pattern components may have affected beetle survival
rates in a manner similar to that of elytral colour luminance (figure 2a, 3), which was a
major component of background-matching camouflage.

(b) Evolution of adaptive colouration

Despite the potential importance of body surface brightness in thermoregulation [78,79],
our PGLS analysis detected no significant effects of climatic factors on colour pattern
evolution (figure 4). This result emphasises the importance of selection pressure for
camouflage as a factor contributing to substantial variation in colour pattern.

Thermoregulation can be accomplished by various behaviours [78,80] that can surpass
the effect of colouration [80]. Behaviours promoting thermoregulation (e.g., basking or
shading) are commonly observed in various tiger beetle species including *C. laetescrpta*
[27,29,45,81]. Therefore, *C. laetescrpta* may rely on behavioural thermoregulation;
selection on body colour for thermoregulatory function may be negligible.

Although *C. laetescrpta* occurs widely in continental Asia, it shows pronounced
elytral colour variation only among Japanese populations [33,34]. Therefore, it is likely
that colour variation originated in this species after its colonisation of Japan from
continental Asia, which is presumed to have occurred during the early to middle Pleistocene [82,83]. We examined population genetic structure and found a significant isolation-by-distance pattern [68] and correlation between geographic and genetic distances (figure 4b), but not between phenotypic and genetic distances (figure 4c). These results suggest that dispersal and colonisation have occurred somewhat gradually (i.e., stepping-stone dispersal) regardless of habitat sand colour, although local adaptation can restrict the dispersal of individuals to ecologically dissimilar habitats [25,84]. In C. laetescripta, colonisation success may have primarily depended on factors other than sand colour, such as the presence of suitable microhabitats for the larval stage [85]; the evolution of observed patterns of colour variation may have followed colonisation under selection pressure from visual predation on elytral colour. Although previous studies of cryptic colouration have investigated clinal variation among populations spanning less than a few hundred km [86,87], our study focused on populations with discontinuous colour variation scattered over an area 1400 km in width (figure 1a). Thus, the findings of our study demonstrate that local adaptation has played a major role in the evolution of background-matching colouration across a wide geographic range of a species. Similar cases of locally fine-tuned camouflage have been suggested in other tiger beetles with wide geographic ranges [69,88].

5. Conclusion

In this study, we applied integrated image analysis, field experiments, and phylogenetic analysis to demonstrate that selection via predation has driven the evolution of finely tuned background-matching camouflage in the tiger beetle C. laetescripta, following the colonisation of different habitats. Our results showed that fine-tuned background matching could be achieved by an evolutionary change in body colour driven by natural selection from visual predators, providing new insight into the evolutionary process and consequences of animal camouflage. These findings will deepen our understanding of the generation and maintenance of great diversity in organismal appearance in nature.
We followed the ethical guidelines for the treatment of animals in the study of animal behaviour [89] in our field predation experiments.

The raw RAD sequence data are deposited at DNA Data Bank of Japan (DDBJ): BioProject accession number PRJDB10308 (SAMD00239266–SAMD00239329).

N.Y. and T.S. conceived the project; N.Y. performed sampling, experiments and data analyses; N.Y. and T.S. wrote the manuscript.

We declare we have no competing interests.

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Figure legends

Figure 1. (a) Sampling sites, sand colouration and elytral colour pattern of the tiger beetle *Chaetodera laetescrpta*. (b) Resemblance of *C. laetescrpta* body colour to sand background at Site F (left) and Site K (right). Photographs by N. Yamamoto.
Figure 2. (a) Correlation of luminance between elytra of *Chaetodera laetescripta* and the sand of its habitats, from the perspective of avian predator vision. Error bars indicate standard deviation. Grey lines are contour lines for given JND values. (b) Correlation between the mean percentage of white area and luminance among *C. laetescripta* elytra.
Figure 3. Percentages of paper tiger beetle models attacked by birds on light sand (left; Site J) and dark sand (right; Site F) during the experiments \((n = 2981)\).
Figure 4. (a) Maximum-likelihood tree of restriction site-associated DNA (RAD) sequences. See electronic supplementary material, table S1 for details of samples. (b) Relationships of genetic distance ($F_{ST}$) with geographic distance and (c) difference in mean percentage of white area in the elytra of *Chaetodera laetescripta*. (d) Mean percentage of elytral white area in the elytra of *C. laetescripta* from each locality (right), with phylogenetic relationships among populations (left).
Table 1. Effects of mean sand luminance (Lum) and climatic environmental factors on the proportion of white elytral area of *Chaetodera laetescrpta*. Temp, mean temperature (°C) in June–September; Radi, mean total global radiation (MJ m\(^{-2}\)) in June–September.

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</tbody>
</table>

**\(P < 0.01\), *\(P < 0.05\)