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**SUPPORTING INFORMATION. Appendix1.**

**The roles of geography, climate and sexual selection in driving divergence among insect populations in mountaintops**

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**TABLE S1.1** Characteristics of the study populations.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Massif** | **Population** | **Elevation****(m a.s.l.)** | **Tmed****(ºC)** | **Tmin****(ºC)** | **Tmax****(ºC)** | **Sunshine****(kJ∙m-2∙dia-1)** |
| 1 | Eastern | Andara | 1900 | 5.128 | -0.985 | 10.994 | 1907.750 |
| 2 | Eastern | Andara casetón | 1660 | 6.248 | 0.206 | 12.026 | 1643.591 |
| 3 | Central | Camburero | 1486 | 6.596 | 0.622 | 12.312 | 1502.519 |
| 4 | Ponga | Campigüeños | 1745 | 5.640 | -0.767 | 14.675 | 1989.653 |
| 5 | Western | Cabroneru | 1990 | 5.312 | -0.753 | 10.981 | 1782.340 |
| 6 | - | Cantu l'osu | 1763 | 5.776 | -0.635 | 11.935 | 1843.680 |
| 7 | Western | Cotalba | 1970 | 4.965 | -1.124 | 10.613 | 1915.544 |
| 8 | Eastern | Hierru | 2430 | 3.251 | -2.986 | 9.117 | 1728.464 |
| 9 | Central | Liordes | 1950 | 4.599 | -1.561 | 10.421 | 2025.599 |
| 10 | Ponga | Llambria | 1737 | 6.200 | -0.440 | 12.133 | 1384.689 |
| 11 | Ponga | Maciedome | 1912 | 5.543 | -0.768 | 11.635 | 1769.315 |
| 12 | Central | Navarros | 2603 | 2.075 | -4.214 | 7.974 | 1976.152 |
| 13 | Central | Peña Castil | 2370 | 3.100 | -3.100 | 8.985 | 1769.117 |
| 14 | Colláu Zorru | Peña Ten | 1800 | 4.350 | -1.880 | 10.340 | 1593.128 |
| 15 | Colláu Zorru | Pileñes | 1981 | 4.986 | -1.247 | 10.975 | 1836.704 |
| 16 | Eastern | Rasa | 2280 | 3.779 | -2.449 | 9.602 | 2028.617 |
| 17 | Ponga | Tiatordos | 1918 | 5.423 | -0.919 | 11.459 | 1693.122 |
| 18 | Western | Traviesos | 2395 | 3.238 | -2.960 | 8.968 | 2127.562 |
| 19 | Central | Uriellu | 1750 | 4.436 | -1.708 | 10.190 | 1667.147 |
| 20 | Western | Vega Ario | 1625 | 6.100 | 0.208 | 11.773 | 2073.555 |
| 21 | Western | Vega Huerta | 2138 | 4.000 | -2.153 | 9.800 | 2006.488 |
| 22 | Western | Vegarredonda | 1465 | 7.051 | 1.135 | 12.663 | 1881.325 |
| 23 | Central | Vidrio | 2119 | 3.473 | -2.749 | 9.314 | 1902.867 |

Tmed: mean annual temperature; Tmin: mean monthly minimum temperature; Tmax: mean monthly maximum temperature; Sunshine: this parameter represents a measure of the potential radiation input reaching the soil in standard and uniform atmospheric conditions (Ninyerola et al. 2005).

**TABLE S1.2** Biometry (mean trait value ± SD) of the study populations. The number of individuals measured is shown in brackets.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Population** | **Femur Length (mm)** | **Pronotal Length (mm)** | **Total Body Length (mm)** | **Wing Length (mm)** |
|  |  | Males | Females | Males | Females | Males | Females | Males | Females |
| 1 | Andara | 7.875 ± 0.158 (6) | 9.821 ± 0.374 (21) | 2.792 ± 0.232 (6) | 3.649 ± 0.211 (21) | 13.813 ± 0.847 (6) | 20.048 ± 1.300 (21) | 7.938 ± 0.282 (6) | 7.643 ± 0.608 (21) |
| 2 | Andara casetón | 8.034 ± 0.437 (11) | 9.842 ± 0.545 (30) | 2.909 ± 0.231 (11) | 3.725 ± 0.242 (30) | 14.239 ± 1.875 (11) | 18.958 ± 2.081 (30) | 8.250 ± 0.727 (11) | 7.754 ± 0.650 (30) |
| 3 | Camburero | 8.125 ± 0 (1) | 10.375 ± 0 (1) | 2.938 ± 0.265 (2) | 3.750 ± 0 (1) | 12.000 ± 0.707 (2) | 17.500 ± 0 (1) | 8.375 ± 0.707 (2) | 8.500 ± 0 (1) |
| 4 | Campigüeños | 8.875 ± 0.177 (4) | 11.286 ± 0.425 (7) | 3.156 ± 0.213 (4) | 4.054 ± 0.296 (7) | 14.344 ± 0.449 (4) | 20.750 ± 1.584 (7) | 9.063 ± 0.657 (4) | 9.089 ± 0.519 (7) |
| 5 | Cabroneru | 7.625 ± 0.177 (2) | 10.292 ± 0.351 (6) | 2.857 ± 0.244 (7) | 3.781 ± 0.170 (12) | 13.911 ± 0.886 (7) | 18.948 ± 0.945 (12) | 8.268 ± 0.532 (7) | 8.271 ± 0.479 (12) |
| 6 | Cantu l'osu | 9.973 ± 0.353 (10) | 11.712 ± 0.399 (10) | 3.548 ± 0.176 (10) | 4.411 ± 0.108 (10) |  |  | 9.849 ± 0.459 (10) | 10.151 ± 0.518 (10) |
| 7 | Cotalba | 8.547 ± 0.291 (8) | 10.858 ± 0.279 (18) | 3.025 ± 0.211 (10) | 4.000 ± 0.199 (20) | 14.588 ± 1.835 (10) | 19.382 ± 1.534 (19) | 8.550 ± 0.446 (10) | 8.494 ± 0.590 (20) |
| 8 | Hierru | 8.513 ± 0.201 (7) | 10.776 ± 0.079 (3) | 3.116 ± 0.097 (8) | 3.973 ± 0.137 (3) |  |  | 8.065 ± 0.499 (8) | 7.991 ± 0.158 (3) |
| 9 | Liordes | 7.375 ± 0 (1) | 9.313 ± 0.140 (5) | 2.500 ± 0 (1) | 3.325 ± 0.112 (5) | 13.125 ± 0 (1) | 20.025 ± 1.073 (5) | 8.000 ± 0 (1) | 7.925 ± 0.381 (5) |
| 10 | Llambria | 9.214 ± 0.267(7) | 10.875 ± 0 (1) | 3.214 ± 0.061 (7) | 4.125 ± 0 (1) | 13.911 ± 0.790 (7) | 20.000 ± 0 (1) | 9.268 ± 0.447 (7) | 10.000 ± 0 (1) |
| 11 | Maciedome | 9.308 ± 0.278 (13) | 11.792 ± 0.523 (9) | 3.250 ± 0.169 (13) | 4.250 ± 0.125 (9) | 12.865 ± 0.832 (13) | 19.861 ± 1.897 (9) | 8.952 ± 0.708 (13) | 9.236 ± 0.588 (9) |
| 12 | Navarros | 7.808 ± 0 (1) | 9.315 ± 0.112 (4) | 2.740 ± 0 (1) | 3.493 ± 0.079 (4) |  |  | 7.671 ± 0 (1) | 7.295 ± 0.505 (4) |
| 13 | Peña Castil | 8.063 ± 0.072 (4) | 9.821 ± 0.426 (7) | 2.625 ± 0.102 (4) | 3.531± 0.265 (8) | 12.875 ± 0.510 (4) | 17.281 ± 1.372 (8) | 7.969 ± 0.780 (4) | 7.422 ± 0.467 (8) |
| 14 | Peña Ten | 8.300 ± 0.371 (5) | 10.036 ± 0.567 (7) | 2.975 ± 0.185 (5) | 3.554 ± 0.456 (7) | 13.925 ± 0.860 (5) | 18.982 ± 1.574 (7) | 8.700 ± 0.314 (5) | 8.304 ± 0.483 (7) |
| 15 | Pileñes | 8.917 ± 0.315 (3) | 10.979 ± 0.496 (6) | 3.208 ± 0.144 (3) | 3.354 ± 0.347 (5) | 14.167 ± 0.520 (3) | 18.854 ± 1.133 (6) | 8.708 ± 0.439 (3) | 8.521 ± 0.390 (6) |
| 16 | Rasa | 7.844 ± 0.359 (4) | 9.766 ± 0.470 (8) | 2.786 ± 0.112 (7) | 3.656 ± 0.248 (8) | 12.411 ± 0.336 (7) | 18.188 ± 0.984 (8) | 7.714 ± 0.248 (7) | 7.438 ± 0.534 (8) |
| 17 | Tiatordos | 8.775 ± 0.296 (15) | 10.985 ± 0.449 (34) | 3.125 ± 0.245 (15) | 3.932 ± 0.194 (37) | 13.825 ± 0.838 (15) | 20.149 ± 1.191 (37) | 8.650 ± 0.545 (15) | 8.932 ± 0.581 (37) |
| 18 | Traviesos | 7.295 ± 0.367 (11) | 9.096 ± 0.443 (26) | 2.635 ± 0.105 (12) | 3.347 ± 0.241 (27) | 12.563 ± 0.919 (10) | 17.302 ± 1.572 (24) | 7.167 ± 0.629 (12) | 7.477 ± 0.398 (27) |
| 19 | Uriellu | 7.600 ± 0.376 (15) | 9.474 ± 0.593 (17) | 2.692 ± 0.155 (15) | 3.493 ± 0.298 (17) | 12.891 ± 0.689 (8) | 18.867 ± 2.399 (15) | 7.741 ± 0.547 (14) | 7.301 ± 0.880 (17) |
| 20 | Vega Ario | 7.969 ± 0.514 (4) | 10.070 ± 0.534 (8) | 2.875 ± 0.323 (4) | 3.578 ± 0.443 (8) | 14.500 ± 0 (1) | 21.075 ± 2.087 (5) | 8.125 ± 0.545 (3) | 7.719 ± 1.193 (8) |
| 21 | Vega Huerta | 7.688 ± 0.234 (6) | 9.068 ± 0.590 (11) | 2.896 ± 0.255 (6) | 3.295 ± 0.346 (11) | 14.021 ± 0.768 (6) | 17.578 ± 1.259 (8) | 7.958 ± 0.660 (6) | 7.693 ± 0.736 (11) |
| 22 | Vegarredonda | 8.644 ± 0.303 (10) | 11.074 ± 0.370 (11) | 2.913 ± 0.167 (10) | 3.864 ± 0.181 (11) | 14.225 ± 1.174 (10) | 19.534 ± 1.451 (11) | 8.575 ± 0.359 (10) | 8.977 ± 0.418 (11) |
| 23 | Vidrio | 7.984 ± 0.360 (7) | 9.949 ± 0.206 (8) | 2.916 ± 0.104 (7) | 3.767 ± 0.164 (8) |  |  | 7.789 ± 0.573 (7) | 7.209 ± 0.663 (8) |

**TABLE S1.3** Acoustic characteristics of male calling songs (mean ± SD) per study population, together with sample size (N).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **N** | **Chirp duration (s)** | **Syllables per chirp (no.)** | **Syllable duration (s)** |
| 1 | Andara casetón  | 4 | 0.099 ± 0.013 | 5.750 ± 1.455 | 0.0062 ± 0.0009  |
| 2 | Camburero | 1 | 0.1161 | 3.0000  | 0.0192  |
| 3 | Llambria  | 3 | 0.227 ± 0.096 | 15.467 ± 2.052 | 0.0062 ± 0.0022 |
| 4 | Maciedome  | 9 | 0.191 ± 0.024  | 14.667 ± 3.048 | 0.0063 ± 0.0006 |
| 5 | Peña Ten | 1 | 0.1653  | 9.200  | 0.0091 |
| 6 | Rasa  | 12 | 0.089 ± 0.018  | 5.150 ± 1.248  | 0.0056 ± 0.0011  |
| 7 | Tiatordos | 12 | 0.206 ± 0.061  | 10.900 ± 5.598 | 0.0069 ± 0.0017  |
| 8 | Traviesos  | 11 | 0.114 ± 0.021 | 8.200 ± 1.628  | 0.0062 ± 0.0009 |
| 9 | Uriellu | 1 | 0.113 | 3.400 | 0.0100  |
| 10 | Vegarredonda | 6 | 0.1559 ± 0.031  | 11.200 ± 2.583  | 0.0074 ± 0.0012  |

**TABLE S1.4** Values of the potential of individual coding (PIC\*) per acoustic trait obtained in 10 individuals (two different songs per individual) at one of the study populations.

|  |  |  |  |
| --- | --- | --- | --- |
|  | CVb | CVi | PIC |
| Chirp duration  | 27.759 | 9.356 | 2.967 |
| Syllables per chirp  | 52.364 | 16.916 | 3.096 |
| Syllable duration | 20.684 | 13.524 | 1.530 |
| Pulses per syllable | 18.329 | 24.835 | 0.738 |

\*We calculated the coefficient of variation (CV = (SD/mean) x 100) for each acoustic parameter within (CVi, mean value) and between individuals (CVb). A measure of call individuality was obtained from the ratio CVb/CVi, which expresses the potential for individual coding (PIC) per parameter. A PIC value greater than one for a given parameter suggests that it may be useful for individual recognition, because intra-individual variability is less than inter-individual variability (see Palmero et al. 2012 and references therein).

References

Palmero, A. M., Illera, J. C., & Laiolo, P. (2012). Song characterization in the spectacled warbler (*Sylvia conspicillata*): a circum-Mediterranean species with a complex song structure. *Bioacoustics, 21,* 175-191.

**TABLE S1.5** Partitioning of variance (%) for genetic, morphologic and acoustic data of populations of *Chorthippus cazurroi* in the Cantabrian Mountains.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Genetics | Femur length | Syllables per chirp (No.) | Syllable duration |
| *Source of variation* |  | *males* | *females* |  |  |
| Among massifs | **95.30** | **45.09** | **41.62** | **45.83** | 2.15 |
| Among populations within massifs | **3.11** | 32.30 | **35.58** | **11.97** | 13.00 |
| Within populations | 1.59 | 22.60 | 22.80 | 42.19 | 84.85 |

Bold: P < 0.05

**TABLE S1.6** Correlation coefficients (r) and statistical significance (p) of (1) Mantel tests relating genetic (GEN), geographic (GEO), ecological (ECO), morphological (MORF) and acoustic (ACOU) distance matrices, and (2) partial Mantel tests controlling for the effect of geographic and ecological distances. Numbers in brackets indicate the number of populations for which distances matrices were calculated. Male and female data are cumulated for genetic data, separated for morphology and only males were analysed for acoustics.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Males + Females | Males | Females |
|  | *r* | *p* | *r* | *p* | *r* | *p* |
| **1) Mantel test** |  |  |  |  |  |  |
| GEN ~ GEO (23) | 0.290 | **0.003** |  |  |  |  |
| GEN ~ ECO (23) | -0.134 | 0.934 |  |  |  |  |
| GEO ~ ECO (23) | 0.037 | 0.271 |  |  |  |  |
| MORF ~ GEN (23) |  |  | 0.028 | 0.384 | 0.016 | 0.412 |
| MORF ~ GEO (23) |  |  | 0.479 | **< 0.001** | 0.456 | **< 0.001** |
| MORF ~ ECO (23) |  |  | 0.121 | 0.130 | 0.233 | **0.019** |
| ACOU ~ GEN (10) |  |  | -0.086 | 0.585 |  |  |
| ACOU ~ GEO (10) |  |  | 0.459 | **0.003** |  |  |
| ACOU ~ ECO (10) |  |  | -0.063 | 0.595 |  |  |
| ACOU ~ MORF (10) |  |  | 0.453 | **0.008** |  |  |
|  |  |  |  |  |  |  |
| **2) Partial Mantel test** |  |  |  |  |  |  |
| MORF ~ GEN ± GEO (23) |  |  | -0.132 | 0.906 | -0.137 | 0.955 |
| MORF ~ GEN ± ECO (23) |  |  | 0.045 | 0.349 | 0.048 | 0.289 |
| ACOU ~ GEN ± GEO (10) |  |  | -0.148 | 0.813 |  |  |
| ACOU ~ GEN ± ECO (10) |  |  | -0.001 | 0.486 |  |  |
| ACOU ~ MORF ± GEO (10) |  |  | 0.372 | **0.033** |  |  |
| ACOU ~ MORF ± ECO (10) |  |  | 0.498 | **0.003** |  |  |

Geography is tested after *log* transforming the Euclidean geographical distance matrix.

Bold: P < 0.05

**TABLE S1.7** Relationships between morphological and acoustic traits of males from 10 different populations. Linear mixed models were run entering the population as a random factor.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Pronotum length | Wing length | Total body length | Peg number | Length of the femur portion with pegs | Chirp duration | Syllables per chirp | Syllable duration |
| Femur length | t48 = 4.730**p < 0.001** | t48 = 3.217**p = 0.002** | t38 = 1.259p = 0.216 | t44 = 6.818**p < 0.001** | t47 = 4.003**p < 0.001** | t47 = 4.003**p < 0.001** | t48 = 2.114**p = 0.040** | t48 = 1.044p = 0.302 |
| Pronotum length |  | t49 = 3.741**p < 0.001** | t39 = 3.256**p = 0.002** | t44 = 1.245p = 0.220 | t44 = 4.123**p < 0.001** | t48 = 0.666p = 0.508 | t49 = -1.303p = 0.199 | t49 = -0.622p = 0.537 |
| Wing length |  |  | t39 = 2.460**p = 0.018** | t44 = 1.644p = 0.107 | t44 = 3.815**p < 0.001** | t48 = 1.379p = 0.174 | t49 = 0.937p = 0.353 | t49 = 0.225p = 0.823 |
| Total body length |  |  |  | t34 = -0.311p = 0.757 | t34 = 1.949p = 0.060 | t38 = 1.151p = 0.257 | t39 = 1.013p = 0.317 | t39 = 0.645p = 0.522 |
| Pegs number |  |  |  |  | t44 = 6.481**p < 0.001** | t43 = 0.446p = 0.657 | t44 = 2.860**p = 0.006** | t44 = 0.671p = 0.506 |
| Length of the femur portion with pegs |  |  |  |  |  | t43 = 2.155**p =** **0.037** | t44 = 2.043**p =** **0.047** | t44 = 0.991p = 0.327 |
| Chirp duration |  |  |  |  |  |  | t48 = 6.946**p < 0.001** | t48 = 4.562**p < 0.001** |
| Syllables per chirp |  |  |  |  |  |  |  | t49 = 0.372p = 0.711 |

Bold: P < 0.05

**TABLE S1.8.** Results of ANOVA testing the effect of population in male acoustic characteristics.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Chirp duration (s) | Syllables per chirp (No.) | Syllable duration (s) |
|  | *df* | *F* | *p* | *F* | *p* | *F* | *p* |
| Population  | 3 | 12.98 | **<0.001** | 17.7 | **<0.001** | 4.089 | **0.016** |
| Residuals | 26 |  |  |  |  |  |  |

Bold: P < 0.05

**TABLE S1.9.** Results of generalized linear models testing the effect of crossing type (three levels: AA, RR, AR+RA) on occurrence of reproduction (yes/no) and reproductive output (total number eggs, hatched eggs and emerged adults) controlling for female life span. Only the two populations in the Eastern massif were considered. The AA crossing is the reference level of analyses and results of AA vs. AR+RA, and AA vs. RR, are shown.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Occurrence of Reproduction | Egg No. | Hatched egg No. | Emerged adult No. |
|  | *t* | *p* | *p(ANOVA)* | *z* | *p* | *p(ANOVA)* | *z* | *p* | *p(ANOVA)* | *z* | *p* | *p(ANOVA)* |
| **Eastern massif** |  |  |  |  |  |  |  |  |  |  |  |  |
| Intercept  | -1.526 | 0.387 |  | 8.721 | **<0.001** |  | 3.145 | **0.002** |  | 1.357 | 0.175 |  |
| Female life span | 0.195 | **0.019** | **0.002** | 4.551 | **<0.001** | **<0.001** | 0.084 | 0.933 | 0.475 | 0.569 | 0.569 | 0.489 |
| Crossing type: AR+RA | -2.487 | 0.167 | 0.206 | -1.692 | 0.091 | **0.021** | -2.116 | **0.034** | 0.072 | -0.827 | 0.408 | 0.718 |
| Crossing type: RR | -2.644 | 0.149 | -2.731 | **0.006** | -1.721 | 0.085 | -0.205 | 0.837 |

Bold: P < 0.05

**FIGURE S1.1** Distance between male and female in different crosses according to the original population of individuals of *Chorthippus cazurroi* in the Cantabrian Mountains (AA: bajo Andara female x bajo Andara male, AR: bajo Andara female x Rasa male, RA: Rasa female x bajo Andara male, RR: Rasa female x Rasa male, VV: Vegarredonda female x Vegarredonda male, VT: Vegarredonda female x Traviesos male; TV: Traviesos female x Vegarredonda male, TT: Traviesos female x Traviesos male). The number of pairs per crossing type is shown inside boxes. For the Eastern massif, differences between crossing types (F3,47 = 4.98, p = 0.004) are shown with different letters; no differences were found in Western massif (F3,48 = 1.92, p = 0.139).



**FIGURE S1.2** Residuals of variables of reproductive output after controlling for female life span in different crosses according to the original population of individuals from the Eastern massif (a, b, c) and the Western massif (d, e, f) in the Cantabrian Mountains. Crosses were: AA: bajo Andara female x bajo Andara male, AR: bajo Andara female x Rasa male, RA: Rasa hfemale x bajo Andara male, RR: Rasa female x Rasa male VV: Vegarredonda female x Vegarredonda male, VT: Vegarredonda female x Traviesos male; TV: Traviesos female x Vegarredonda male, TT: Traviesos female x Traviesos male. The number of pairs per crossing type is shown inside boxes. Differences among crossing types are shown with different letters only for the Eastern massif at p<0.05.



**FIGURE S1.3** Network of Cytochrome oxidase subunit I sequences found in *Chorthippus cazurroi* along its whole range of distribution. Coloured circles depict unique haplotypes, and size is proportional to their abundance. Black dots represent the number of substitutions needed to change between haplotypes.

