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Transitions between phases of genomic differentiation during stick-insect speciation

Rüdiger Riesch[1†], Moritz Muschick[2†], Dorothea Lindtke[3†], Romain Villoutreix[3†], Aaron A. Comeault[4], Timothy E. Farkas[5], Kay Lucek[3], Elizabeth Hellen[3], Víctor Soria-Carrasco[3], Stuart R. Dennis[6], Clarissa F. de Carvalho[3], Rebecca J. Safran[7], Cristina P. Sandoval[8], Jeff Feder[9], Regine Gries[10], Bernard J. Crespi[10], Gerhard Gries[10], Zach Gompert[11†] and Patrik Nosil[3]*

4 **Updating the *T. cristinae* reference genome with additional linkage mapping.** We generated

5 GBS data to improve (relative to[1]) the clustering of scaffolds into linkage groups (LGs) for the *T.*

6 *cristinae* reference genome. In particular, we generated two additional lanes of Illumina hi-seq

7 DNA sequence data with V3 reagents (128 million high-quality sequences) for individuals from

8 three mapping families (192 individuals), which we combined with the data reported in[1]. We

9 aligned these data to the reference genome scaffolds using BWA 0.7.5a-r405[2] (the aln and samse

10 algorithms) with a maximum number of allowed mismatches of 4, and a minimum base quality

11 score of 10. We placed only sequences with a unique best hit. We then used the Bayesian variant

12 caller in SAMTOOLS and BCFTOOLS 0.1.19 to identify variable nucleotides and to calculate

13 genotype likelihoods based on the combined new and previous data[1]. We performed variant

14 calling separately for each family with minimum base and alignment quality scores of 15 and 10,

15 respectively, data required for 80% or more of the individuals in a family, and the full prior with

16 θ set to 0.001. We called variants only if the posterior probability of the data under the null

17 model of no variation was less than 0.001. We then estimated recombination rates between all

18 pairs of SNPs within each family, as previously described[1]. We included data only from

19 individuals and loci where the posterior probability of the most likely genotype was 0.95

20 (offspring posterior probabilities were calculated using the genotype likelihoods from BCFTOOLS

21 and a prior based on the parental genotypes and Mendelian inheritance; see[1] for details). We then

22 constructed LGs from the recombination rate estimates using a heuristic clustering algorithm, as

23 described in[1]. Overall the new LGs were similar to those generated previously. In particular, it

24 was highly correlated between assemblies whether scaffolds were on the same or different LGs (r

25 = 0.8). We treat the resulting new LG designation and ordering on the new draft genome (v0.3)

26 as our best working hypothesis for the genome organization of *T. cristinae* that will iteratively be

27 improved over time. The new draft genome (v0.3) includes 1413 scaffolds assigned to 13 LGs

28 (551 Mb of the genome were assigned to linkage groups [53%]), with an average of 109

29 scaffolds per linkage group.

30

31 **Sampling survey of *Timema* populations across California to study stages of speciation.** In

1   April and May 2012, we obtained *Timema* samples at 47 geographic sites across California, and

2   stored them in 96% ethanol. Detailed information on samples is given in Supplementary Table 1

3   and Supplementary Fig. 4 at the bottom of this document.

4

5   **Supplementary Table 1.** Details about the populations of 12 Californian *Timema* (*T.*) species

6   sampled. Number of individuals refer to the total number of individuals sampled and include

7   those discarded for further analyses because of a low number of sequence reads.

Species	Locality	Latitude (N)	Longitude (W)	Host code	Host	No individuals
T. bartmani	BMCG3	33.83	-116.74	IC	*Calocedrus decurrens*	1
T. bartmani	BMCG3	33.83	-116.74	WF	*Abies concolor*	20
T. bartmani	BMP90	33.80	-116.70	P	*Pinus sp.*	40
T. bartmani	BMP90	33.80	-116.70	WF	*Abies concolor*	1
T. bartmani	BMPCT	33.84	-116.74	IC	*Calocedrus decurrens*	1
T. bartmani	BMPCT	33.84	-116.74	WF	*Abies concolor*	39
T. bartmani	JL	34.16	-116.90	P	*Pinus sp.*	20
T. bartmani	JL	34.16	-116.90	WF	*Abies concolor*	20
T. bartmani	PCT8000ft	33.83	-116.72	P	*Pinus sp.*	15
T. bartmani	PCTCR	33.83	-116.71	P	*Pinus sp.*	19
T. bartmani	PCTCR	33.83	-116.71	WF	*Abies concolor*	19
T. boharti	SRTH	32.98	-116.52	C	*Ceanothus sp.*	8
T. californicum	LICK	37.34	-121.65	Q	*Quercus sp.*	20
T. californicum	LP	37.10	-121.88	Q	*Quercus sp.*	20
T. californicum	SM	37.02	-121.73	M	*Arctostaphylos sp.*	17
T. californicum	SM	37.02	-121.73	Q	*Quercus sp.*	20
T. chumash	BALD	34.22	-117.67	C	*Ceanothus sp.*	7
T. chumash	BALD	34.22	-117.67	MM	*Cercocarpus betuloides*	4
T. chumash	BALD	34.22	-117.67	Q	*Quercus sp.*	7
T. chumash	BMT	33.83	-116.80	C	*Ceanothus sp.*	20
T. chumash	BMT	33.83	-116.80	Q	*Quercus sp.*	15
T. chumash	BS	33.82	-116.79	C	*Ceanothus sp.*	20
T. chumash	BS	33.82	-116.79	Q	*Quercus sp.*	20
T. chumash	DZ243	33.86	-116.83	M	*Arctostaphylos sp.*	20
T. chumash	GR104	34.23	-117.68	Q	*Quercus sp.*	20
T. chumash	GR603	34.22	-117.74	Q	*Quercus sp.*	17

T. chumash	GR806	34.22	-117.71	MM	*Cercocarpus betuloides*	16
T. chumash	GR806	34.22	-117.71	Q	*Quercus sp.*	20
T. chumash	HF4	34.27	-118.10	C	*Ceanothus sp.*	1
T. chumash	HF4	34.27	-118.10	Q	*Quercus sp.*	7
T. chumash	HF6	34.27	-118.12	Q	*Quercus sp.*	5
T. chumash	HFRBP	34.26	-118.11	M	*Arctostaphylos sp.*	21
T. chumash	HFRBP	34.26	-118.11	Q	*Quercus sp.*	20
T. chumash	HFRS	34.36	-118.01	M	*Arctostaphylos sp.*	20
T. chumash	HFRS	34.36	-118.01	MM	*Cercocarpus betuloides*	20
T. chumash	HFRS	34.36	-118.01	Q	*Quercus sp.*	20
T. chumash	HFTP	34.34	-117.98	C	*Ceanothus sp.*	18
T. chumash	PF243	33.86	-116.84	A	*Adenostoma fasciculatum*	1
T. chumash	PF243	33.86	-116.84	C	*Ceanothus sp.*	20
T. chumash	PF243	33.86	-116.84	M	*Arctostaphylos sp.*	14
T. chumash	PF243	33.86	-116.84	Q	*Quercus sp.*	5
T. cristinae	BY	34.50	-119.86	A	*Adenostoma fasciculatum*	20
T. cristinae	BY	34.50	-119.86	C	*Ceanothus sp.*	20
T. cristinae	BY	34.50	-119.86	MM	*Cercocarpus betuloides*	20
T. cristinae	BY	34.50	-119.86	Q	*Quercus sp.*	10
T. cristinae	ECCAMP	34.51	-119.76	A	*Adenostoma fasciculatum*	19
T. cristinae	ECCAMP	34.51	-119.76	M	*Arctostaphylos sp.*	20
T. cristinae	ECCAMP	34.51	-119.76	Q	*Quercus sp.*	20
T. cristinae	OUT	34.53	-119.84	A	*Adenostoma fasciculatum*	3
T. cristinae	OUT	34.53	-119.84	C	*Ceanothus sp.*	2
T. cristinae	R23	34.52	-120.08	A	*Adenostoma fasciculatum*	20
T. cristinae	R9	34.51	-120.07	C	*Ceanothus sp.*	7
T. cristinae	VP	34.53	-119.85	C	*Ceanothus sp.*	20
T. cristinae	VP	34.53	-119.85	M	*Arctostaphylos sp.*	4
T. cristinae	VP	34.53	-119.85	Q	*Quercus sp.*	20
T. sp. 'cuesta ridge'	CR	35.36	-120.65	A	*Adenostoma fasciculatum*	20
T. sp. 'cuesta ridge'	CR	35.36	-120.65	C	*Ceanothus sp.*	20
T. sp. 'cuesta ridge'	CR	35.36	-120.65	CY	*Cupressus sargentii*	20
T. sp. 'cuesta ridge'	CR	35.36	-120.65	M	*Arctostaphylos sp.*	19

T. sp. 'cuesta ridge'	CR	35.36	-120.65	Q	*Quercus sp.*	6
T. knulli	BCE	36.07	-121.60	RW	*Sequoia sempervirens*	15
T. knulli	BCTUR	36.08	-121.61	C	*Ceanothus sp.*	17
T. knulli	BCTUR	36.08	-121.61	P	*Pinus sp.*	16
T. knulli	BCWP	36.07	-121.60	C	*Ceanothus sp.*	12
T. knulli	BCWP	36.07	-121.60	Q	*Quercus sp.*	1
T. knulli	H1M37	36.17	-121.68	C	*Ceanothus sp.*	4
T. knulli	H1M37	36.17	-121.68	Q	*Quercus sp.*	1
T. knulli	HB	36.16	-121.67	C	*Ceanothus sp.*	20
T. knulli	HB	36.16	-121.67	Q	*Quercus sp.*	3
T. landelsensis	BCBOG	36.07	-121.58	C	*Ceanothus sp.*	23
T. landelsensis	BCBOG	36.07	-121.58	Q	*Quercus sp.*	20
T. landelsensis	BCHC	36.06	-121.57	M	*Arctostaphylos sp.*	3
T. landelsensis	BCHC	36.06	-121.57	Q	*Quercus sp.*	20
T. landelsensis	BCOG	36.07	-121.58	C	*Ceanothus sp.*	5
T. landelsensis	BCOG	36.07	-121.58	Q	*Quercus sp.*	20
T. landelsensis	BCSUM	36.06	-121.56	C	*Ceanothus sp.*	20
T. landelsensis	BCSUM	36.06	-121.56	M	*Arctostaphylos sp.*	3
T. landelsensis	BCSUM	36.06	-121.56	Q	*Quercus sp.*	11
T. petita	101SS	35.73	-121.31	C	*Ceanothus sp.*	20
T. podura	BMCG3	33.83	-116.74	IC	*Calocedrus decurrens*	20
T. podura	BMCG3	33.83	-116.74	Q	*Quercus sp.*	20
T. podura	BME	33.80	-116.76	A	*Adenostoma fasciculatum*	20
T. podura	BME	33.80	-116.76	C	*Ceanothus sp.*	4
T. podura	BMLC	33.81	-116.75	M	*Arctostaphylos sp.*	1
T. podura	BMLC	33.81	-116.75	Q	*Quercus sp.*	20
T. podura	BMOKC	33.82	-116.75	Q	*Quercus sp.*	18
T. podura	BMPCT	33.84	-116.74	IC	*Calocedrus decurrens*	19
T. podura	BMPCT	33.84	-116.74	M	*Arctostaphylos sp.*	1
T. podura	BMPCT	33.84	-116.74	WF	*Abies concolor*	1
T. podura	BMT	33.83	-116.80	C	*Ceanothus sp.*	20
T. podura	BMT	33.83	-116.80	Q	*Quercus sp.*	18
T. podura	BS	33.82	-116.79	C	*Ceanothus sp.*	3
T. podura	BS	33.82	-116.79	Q	*Quercus sp.*	3
T. podura	DZ243	33.86	-116.83	A	*Adenostoma fasciculatum*	20
T. podura	DZ243	33.86	-116.83	M	*Arctostaphylos sp.*	10

T. podura	PCT8000ft	33.83	-116.72	P	*Pinus sp.*	5
T. podura	PCTCR	33.83	-116.71	P	*Pinus sp.*	1
T. podura	PF243	33.86	-116.84	A	*Adenostoma fasciculatum*	20
T. podura	PF243	33.86	-116.84	C	*Ceanothus sp.*	8
T. podura	PF243	33.86	-116.84	M	*Arctostaphylos sp.*	5
T. podura	PF243	33.86	-116.84	Q	*Quercus sp.*	13
T. podura	SRHWY	32.82	-116.51	A	*Adenostoma fasciculatum*	5
T. poppensis	FROCK	38.89	-123.38	C	*Ceanothus sp.*	1
T. poppensis	FROCK	38.89	-123.38	DF	*Pseudotsuga menziesii*	20
T. poppensis	LP	37.10	-121.88	DF	*Pseudotsuga menziesii*	16
T. poppensis	MM	37.00	-121.71	RW	*Sequoia sempervirens*	20
T. poppensis	SM	37.02	-121.73	RW	*Sequoia sempervirens*	19
T. poppensis	TBARN	38.62	-123.29	DF	*Pseudotsuga menziesii*	20
T. poppensis	TBARN	38.62	-123.29	RW	*Sequoia sempervirens*	20
T. shepardi	FROCK	38.89	-123.38	C	*Ceanothus sp.*	12

1

2 **Genotyping-by-sequencing (GBS) and stages of speciation.** We obtained 1,157,803,056

3 Illumina single-end 100 bp reads from 1545 individuals sequenced across seven sequencing

4 lanes on the Hiseq2000 platform, which were parsed using custom Perl scripts based on code

5 from a previous study[3]. We identified and removed the in-line barcodes, including those that

6 were 1 bp away due to synthesizing or sequencing errors, and relabelled the sequences with the

7 corresponding sample identifiers. In addition, we removed the following six base pairs of the

8 EcoRI cut site and the adapters at the 3' end when present. We discarded sequences that were

9 shorter than 16 bp after parsing or those lacking barcodes, as well as all reads of the asexual

10 species *Timema shepardi* (n = 53,569,163). The total number of reads retained for the remaining

11 1533 individuals was 1,104,233,893, and the mean number of reads per individual was 720,309

12 (95% interval = 161,807-1,576,215). The average length of the sequences was 73 bp (95%

13 interval = 67-78 bp). We aligned 71.6% of the reads (790,903,445) to the *T. cristinae* reference

14 genome previously published[1] using BOWTIE2 2.1.0[4] with the local model and the '--very-

15 sensitive-local' preset (-D 20 -R 3 -N 0 -L 20 -i S,1,0.50). The average number of mapped reads

16 per individual was 512,235 (95% interval = 109,824 – 1,166,144).

17

18 Following mapping, we excluded from further analyses an additional 28 individuals that had

1 fewer than 100,000 mapped reads. We used SAMTOOLS 0.1.19[5] to sort and index the alignments

2 of the remaining 1505 individuals, which we used in further analyses. Variants were called using

3 SAMTOOLS mpileup and BCFTOOLS using the full prior and requiring the probability of the data

4 to be less than 0.5 under the null hypothesis that all samples were homozygous for the reference

5 allele to call a variant. We ignored insertion and deletion polymorphisms. We identified 726,955

6 single nucleotide variants (SNVs) with an average depth across all individuals of ~4768x (mean

7 coverage per variant per individual ~ 3×, median coverage ~ 1×). We applied further, more

8 stringent filtering schemes specific to particular downstream analyses, which are described in the

9 corresponding sections below.

10

11 We measured genome-wide genetic differentiation between pairs of populations using the

12 Hudson's F_{ST} estimator[6]. For each population and variant, we inferred maximum-likelihood

13 allele frequencies from the genotype likelihoods by means of the iterative soft expectation-

14 maximization algorithm (EM), as before. We developed a Perl script to calculate Hudson's F_{ST}

15 from bcf files. All the code has been deposited in a Dryad repository at

16 http://dx.doi.org/10.5061/dryad.nq67q. We estimated F_{ST} for every pair of populations (defined

17 as the pool of individuals from the same species, locality, and host plant), but excluded

18 populations with less than two individuals beforehand. We applied a general filter excluding

19 variants that were present in less than 90% of the individuals, had a quality score below 20, or

20 had a depth across individuals above 10,000. For each comparison, we further filtered out the

21 SNVs that were present in less than 50% of the individuals from the two populations and had a

22 pooled MAF estimate below 5%. This resulted in a variable number of variants used for F_{ST}

23 estimation in each comparison, ranging from 126 to 1909 (mean = 1245, median = 1361, 95%

24 interval = 418 – 1769). A table with details about all the comparisons and F_{ST} estimates have

25 been made available in the Dryad repository.

26

27 We also estimated genetic structure and potential admixture using a hierarchical Bayesian model

28 that jointly estimates genotypes and admixture proportions as implemented in the program

29 ENTROPY 1.2b[7]. This model is similar to the popular STRUCTURE[8] algorithm, but accounts for

30 sequencing errors and genotype uncertainties inherent to next-generation sequencing methods.

31 We estimated parameters for a model with K=2 population clusters for every pair of populations

1 found at the same geographic locality but belonging to different species, and for K=number-of-

2 hosts-plants for conspecific populations found at the same geographic locality (Supplementary

3 Table 2). In addition, we fitted a model with K=1 in both cases and evaluated what model fit the

4 data better using the difference in Deviance Information Criterion (ΔDIC, negative values

5 indicate data favours K=1). DIC penalizes model complexity by adding to the posterior mean

6 deviance the effective number of parameters (approximated as half the posterior variance of the

7 deviance) [9]. For each pair, we used the bi-allelic SNVs for which there were sequence data from

8 at least 85% of the samples involved in each comparison and that were not fixed within any of

9 the populations compared. We set the scalar of the Dirichlet initial value of q to 50. As starting

10 admixture proportions, we used values obtained by applying linear discriminant analysis on a

11 covariance matrix of composite genotypes estimated assuming Hardy-Weinberg equilibrium. We

12 ran two independent Markov Chain Monte Carlo (MCMC) analyses for 35,000 generations and

13 took samples every 10th iteration. We assessed mixing and convergence by visually inspecting

14 the posterior deviance traces. We discarded the first 1,000 samples (10,000 iterations) from each

15 chain as a burn-in, and combined the two chains (5,000 samples in total) to estimate model

16 parameters. Details are provided in Supplementary Table 2.

17

18 **Supplementary Table 2.** Details on the number of individuals, single nucleotide variants

19 (SNVs), admixture proportions, and difference in Deviance Information Criterion (ΔDIC)

20 estimated with ENTROPY for conspecific populations on different hosts (among populations) and

21 for different species ignoring hosts (among species), sampled from the same locality in both

22 cases. Admixture proportions are given for a number of clusters K=number-of-hosts-plants for

23 among populations comparisons (only species sampled from 2 or more host plants from the same

24 locality are shown) and K=number of species for among species comparisons. Sample sizes and

25 admixture proportions are showed in the same order than hosts and species. ΔDIC is the

26 difference in DIC between K=1 and the K used for estimating admixture proportions (negative

27 values indicate K=1 is a better fit). Host codes are A for chamise (*Adenostoma fasciculatum*), C

28 for California lilac (*Ceanothus spinosus*), CY for Sargent's cypress (*Cupressus sargentii*), DF

29 for Douglas fir (*Pseudotsuga menziesii*), IC for incense cedar (*Calocedrus decurrens*), LP for

30 lodgepole pine (*Pinus contorta*) , M for manzanita (*Arctostaphylos sp.*), MM for mountain

1   mahogany (*Cercocarpus betuloides*), P for other pine (*Pinus sp.*), Q for oak (*Quercus sp.*), RW

2   for redwood (*Sequoia sempervirens*), and WF for white fir (*Abies alba*).

1

	Locality	Species	Hosts	Sample size	SNVs	Admixture	ΔDIC
	BMCG3	*T. bartmani*	IC,WF	1,19	1138	0.52,0.51	-832
	BMP90	*T. bartmani*	LP,WF,P	20,1,15	1103	0.33,0.21,0.23	651
	BMPCT	*T. bartmani*	IC,WF	1,39	1354	0.70,0.36	700
	JL	*T. bartmani*	WF,P	20,20	783	0.97,0.98	-14616
	PCTCR	*T. bartmani*	WF,P	19,18	1361	0.71,0.28	-14939
	SM	*T. californicum*	M,Q	17,19	1199	0.16,0.53	-16836
	BALD	*T. chumash*	C,MM,Q	7,4,7	1315	0.22,0.20,0.57	-10777794
	BMT	*T. chumash*	C,Q	20,15	678	0.99,0.93	-8143
	BS	*T. chumash*	C,Q	20,19	944	0.03,0.05	-12213
	GR806	*T. chumash*	MM,Q	16,20	2462	0.83,0.64	-12557
	HF4	*T. chumash*	C,Q	1,7	1107	0.17,0.22	-26002
Among populations	HFRBP	*T. chumash*	M,Q	21,20	1827	0.98,0.24	-6890
	HFRS	*T. chumash*	M,MM,Q	20,20,19	919	0.59,0.79,0.38	-96398
	PF243	*T. chumash*	A,C,M,Q	1,19,13,5	693	0.40,0.34,0.30,0.47	-13003
	BY	*T. cristinae*	A,C,MM,Q	20,19,19,10	2739	0.00,0.00,0.00,0.20	-59632
	OUT	*T. cristinae*	A,C	3,2	805	0.50,0.50	-4704
	VP	*T. cristinae*	C,M,Q	20,4,20	1891	0.10,0.18,0.12	-17356
	CR	*T. 'Cuesta Ridge'*	A,C,CY,M,Q	20,18,20,19,6	2114	0.11,0.03,0.03,0.03,0.11	-409688
	BCTUR	*T. knulli*	C,P	17,16	1933	0.59,0.00	9784
	BCWP	*T. knulli*	C,Q	10,1	1223	0.26,0.27	-53569
	H1M37	*T. knulli*	C,Q	4,1	785	0.50,0.57	-32921
	HB	*T. knulli*	C,Q	20,3	1702	0.72,0.72	-230375
	BCBOG	*T. landelsensis*	C,Q	23,20	1913	0.88,0.71	-12760
	BCHC	*T. landelsensis*	M,Q	3,20	1814	0.45,0.62	-15401597
	BCOG	*T. landelsensis*	C,Q	4,18	1597	0.11,0.10	-60971
	BCSUM	*T. landelsensis*	C,M,Q	20,3,11	1657	0.54,0.02,0.54	-37446
	BMCG3	*T. podura*	IC,Q	19,19	1950	0.63,0.52	-10337
	BME	*T. podura*	A,C	20,4	1903	0.95,0.24	-4103
	BMLC	*T. podura*	M,Q	1,20	1898	0.15,0.11	-89279
	BMPCT	*T. podura*	IC,M,WF	19,1,1	2225	0.32,0.00,0.00	-12950
	BMT	*T. podura*	C,Q	20,18	2573	0.49,0.36	-2216
	BS	*T. podura*	C,Q	3,3	1201	0.48,0.49	-26228
	DZ243	*T. podura*	A,M	20,8	2196	0.69,0.57	-15758
	PF243	*T. podura*	A,C,M,Q	20,8,5,13	2335	0.28,0.16,0.37,0.15	-228622
	FROCK	*T. poppensis*	C,DF	1,20	1467	0.01,0.13	-164578
	TBARN	*T. poppensis*	DF,RW	20,20	935	0.48,0.52	1083

2

LP	*T. californicum,* *T. poppensis*	DF,Q	20,16	2152	1.000,0.000	139240
SM	*T. californicum,* *T. poppensis*	M,Q,RW	36,17	2243	1.000,0.000	151576
BMT	*T. chumash,* *T. podura*	C,Q	35,38	4194	0.999,0.000	560870
BS	*T. chumash,* *T. podura*	C,Q	39,6	3105	0.003,1.000	190033
DZ243	*T. chumash,* *T. podura*	A,M	20,28	4098	0.996,0.001	322521
PF243	*T. chumash,* *T. podura*	A,C,M,Q	38,46	4040	1.000,0.000	569373
BMCG3	*T. bartmani,* *T. podura*	IC,Q,WF	20,38	3108	0.000,1.000	160123
BMPCT	*T. bartmani,* *T. podura*	IC,M,WF	40,21	3517	0.000,1.000	187304
PCTCR	*T. bartmani,* *T. podura*	WF,P	37,1	2015	0.998,0.030	-3928

(left margin label: Among species)

1

2 **Maximum-likelihood phylogenetic inference and genealogical sorting index (GSI).** We

3 removed, from the raw dataset of SNVs described above, variants with sequence data for less

4 than 85% of the individuals, a depth greater than 10,000, a phred-scale quality score lower than

5 20, or more than two alleles. We kept 28,701 variants with a mean depth across all individuals of

6 ~6930× (mean coverage per variant per individual ~ 4.5×). We used a custom Perl script to

7 generate a multiple alignment that encoded heterozygous genotypes as IUPAC ambiguities. We

8 partitioned the alignment by linkage group (LG) and excluded the positions in genomic regions

9 not assigned to any linkage group. This resulted in a multiple alignment of a total of 1505

10 individuals and 19,556 positions distributed in 13 partitions as follows: LG1: 1185, LG2: 466,

11 LG3: 4033, LG4: 2866, LG5: 464, LG6: 2097, LG7: 622, LG8: 2030, LG9: 1291, LG10: 1642,

12 LG11: 1273, LG12: 220, LG13: 1367. We inferred 1000 maximum-likelihood bootstrap trees

13 using the rapid heuristic algorithm implemented in RAxML 8.2.9[10,11]. For each partition, we used a

14 GTR substitution model, rate heterogeneity was incorporated using the CAT model with 25

15 categories, and likelihood was corrected for ascertainment bias using the Lewis approach[12].

16 Maximum-likelihood optimizations were started from random starting trees. We performed an *a*

1   *posteriori* bootstrapping analysis using the extended majority-rule consensus (autoMRE)

2   criterion with the recommended cutoff threshold of 0.03. This analysis indicated convergence

3   after 500 bootstrap replicates. We used the R package ape[13] to root bootstrap trees between the

4   North Clade + Santa Barbara Clade and the South Clade (following root placement inferred in

5   Bayesian phylogenetic analyses below). Subsequently, we used the R package

6   genealogicalSorting to calculate, for each bootstrap tree, the Genealogical Sorting Index (GSI)

7   for each of the 166 groups with at least 2 individuals delimited by species (11), species and

8   locality (56), and species, locality, and host (98). Bootstrap trees and tables with all GSI values

9   have been deposited in Dryad.

10

11   **Genotyping-by-sequencing (GBS) for tests on the effect of colour-pattern and CHC on**

12   **genome-wide differentiation.** We sequenced individually barcoded restriction-site associated

13   DNA libraries of 325 samples from 19 *T. cristinae* populations on three Illumina lanes, using

14   molecular and analytical methods described above (section GBS and stages of speciation). We

15   combined these new sequences with 17 randomly chosen samples (10 males and 7 females) from

16   the FHA mapping population, resulting in sequences from 342 individuals spanning 20

17   populations (5-20 individuals per population, mean = 17) for population genetic analyses of

18   genetic differentiation. After filtering raw sequences (minimum read and base quality score 20,

19   minimum read length 50 bp after trimming), we obtained 286,357,541 DNA sequences for all

20   342 samples (mean 837,303 reads per individual with mean read length 83.9 bp). We mapped

21   94.2% (269,643,356) reads to the *T. cristinae* draft genome v0.3a (90.9% of LG designation and

22   ordering identical to new v0.3) using BOWTIE2 2.2.3 with the '--very-sensitive-local' preset. We

23   used SAMTOOLS to sort and index alignments, and identified SNPs with SAMTOOLS mpileup and

24   BCFTOOLS using the full prior and requiring the probability of the data being homozygous for the

25   reference allele to be less than 0.01. We further discarded variants with low quality (score below

26   20) and where less than 90% of samples were covered. We retained 613,261 bi-allelic SNPs with

27   mean coverage depth per SNP per individual ~5× (per SNP average ranging from 2.2 to 28.7; per

28   individual average ranging from 1.0 to 10.3).

29

30   We estimated genome-wide Hudson's F_{ST} for all 190 population pairs as $F_{ST} = 1 - Hw/Hb$. Hw is

31   the mean number of differences among sequences from the same population, and Hb the mean

1   number of differences among sequences from different populations, averaged over loci. We

2   calculated Hw and Hb for each locus from population allele frequencies estimated using

3   genotype probabilities obtained with SAMTOOLS and BCFTOOLS, as in[1]. For each population pair,

4   we excluded loci with a MAF less than 0.05, or where less than 50% of individuals were

5   covered.

6

7   **Whole-genome re-sequencing of 10 population pairs spanning eight species.** We sequenced

8   an additional 384 *Timema* genomes using the same protocols as for *T. cristinae*[1]. Of these, five

9   were not appropriate for population genetic analyses, as they were single specimens each from a

10 single locality. We did not analyse them further here following assembly and variant calling and

11 will use them in future work. The other 379 genomes, which we do analyse here in a population

12 genetic framework, stem from 16-20 individuals per population sampled from 10 parapatric pairs

13 of host-associated populations. We sampled each of these pairs at the same general locality,

14 usually directly adjacent to one another (the one exception was HFRS, where each population

15 was sampled in the same general locality but separated by a slightly larger distance of ~3 km

16 rather than the usual hundreds of metres). As with the genomes from the transplant experiment,

17 we aligned the paired-end sequences to the *T. cristinae* reference genome (v0.3) using the BWA-

18 MEM algorithm in BWA 0.7.5a-r405[2]. We used a minimum seed length of 20 bp, searched for

19 internal seeds in seeds longer than 1.3 * 20 base-pairs, discarded chains if the seeded bases were

20 shorter than 100 bp, and set the minimum score to output an alignment to 30. We then used

21 SAMTOOLS to compress, sort, and index the alignments and to remove potential PCR duplicates.

22 We then identified variant nucleotides using the UnifiedGenotyper in GATK with the prior

23 probability of heterozygosity set to 0.001, a minimum base quality score of 20, a call confidence

24 threshold of 50, and a maximum of 2 alleles allowed. We considered only SNPs that mapped to

25 one of the 13 identified LGs (i.e., due to our interest in genetic architecture we ignored the

26 scaffolds not assigned to a LG). We further filtered the initial set of variants by retaining only

27 those with (*i*) a minimum total sequencing depth of 384, (*ii*) a minimum of 10 reads supporting

28 the non-reference allele, (*iii*) no more than 1% of reads spanning an insertion-deletion, (*iv*) no

29 more than 5 mapping quality 0 reads, (*v*) a maximum absolute value of the base quality rank sum

30 test of 3, (*vi*) a maximum absolute value of the mapping quality rank sum test of 2, (*vii*) a

31 maximum absolute value of the read position rank sum test of 2, and (*viii*) a minimum ratio of

1   the variant confidence score to the non-reference read depth of 2. We then discarded SNPs with a

2   MAF less than 1% (across all individuals), which left us with 5.07 million SNPs for subsequent

3   analyses.

4

5   **Population genetics using whole genomes from eight species.** We obtained maximum

6   likelihood allele frequency estimates for each of the 20 populations (10 population pairs) for

7   each of the 5.07 million SNPs identified above. We did this using an expectation-maximization

8   (EM) algorithm that accounts for uncertainty in the underlying genotypes of individuals and that

9   thus can work directly with the relative genotype likelihoods from GATK's UnifiedGenotyper.

10   We implemented the previously described algorithm[14] in a stand-alone C++ program written

11   using the Gnu Scientific Library. We set the tolerance for EM convergence to 0.001 and the

12   maximum number of EM iterations to 20. We then used these maximum likelihood allele

13   frequency estimates to calculate sequence-based estimates of F_{ST} between each of the 10 pairs of

14   ecotypes, as described above. The set of 10-population pairs included four populations from each

15   of two species (and two populations from the other six). Additionally, Nei's measure of absolute

16   divergence (D_{XY})[15] was determined for each 20-kb window for the two hetero-specific

17   population pairs (LP and SM).

18

19   We used Approximate Bayesian Computation (ABC) to estimate parameters of a Wright-Fisher

20   model with migration to quantify gene flow (the number of migrants per generation or $N_e m$)

21   between the 10 pairs (Supplementary Table 3, Supplementary Figs. 1 and 4). We inferred gene

22   flow from random sets of 5000 SNPs with MAF >5% in order to obtain an estimate of the

23   genome-average effective gene flow for each pair. We assumed discrete generations, where the

24   pair of populations (with constant sizes N_0 and N_1) diverged t generations in the past and has

25   experienced constant, symmetric migration at rate m (where m is the proportion of migrant

26   individuals). Ancestral allele frequencies are fixed at the mean for the pair of populations. We

27   then used ABC to infer t and $N_e m$ (the product of the mean of N_0 and N_1, and the migration rate).

28   We used this approach rather than a coalescent-based ABC analysis or other methods based on

29   diffusion approximations because it is a valid and efficient way to make inferences about recent

30   evolutionary dynamics as in [16]), and does not make the assumption that gene flow is a weak force

31   in contrast to [17]).

2   We placed log uniform priors with lower and upper bounds of 200 and 20,000 on the population

3   sizes, and lower and upper bounds of 10 and 50,000 on the split time between taxon pairs. We

4   placed a uniform prior bounded by 0 and 0.2 on the migration rate. We chose these priors to

5   focus computational efforts on reasonable portions of parameter space. We selected summary

6   statistics that are informative about divergence time and migration rate: a multilocus estimator of

7   F_{ST}, and the proportion of alleles that were rare or absent (MAF < 5%) in one population but not

8   the other (MAF > 5%). We calculated the latter separately for each population in the pair. We

9   then ran 500,000 ABC simulations from the Wright-Fisher model with parameter values sampled

10   from their priors. We wrote the code to conduct the simulations and to calculate the summary

11   statistics in C++ using the Gnu Scientific Library. We then used the rejection method with local-

12   linear adjustment implemented in the R package abc to estimate the posterior distribution for

13   each parameter[18,19]. We retained only the 0.1% of samples with summary statistics closest to the

14   observed values, and we log- (all but m) or logit- (m) transformed the parameters during the

15   inference procedure.

16

17   We detected non-negligible levels of gene flow between most taxon pairs (the primary exception

18   involved the heterospecific species pair; range of N_em for conspecific pairs = 0.24 - 1.82, range

19   of N_em for heterospecific pairs = 0.0067 - 0.0078). As expected, rates of gene flow estimated

20   using ABC declined with average genome-wide F_{ST}. We obtained similar results if we estimated

21   N_em simply based on an equilibrium island model as $Nm = -(F_{ST}-1)/(4F_{ST})$[20].

22

23   **Supplementary Table 3.** Population pairs used for whole genome re-sequencing and their

24   characteristics. (A) Sample information: n1= sample size on host 1, n2 = sample size on host 2.

25   (B) Gene flow estimates (N_em or number of migrants per generation) between 10 taxon pairs of

26   *Timema*. Parameter estimates from the Approximate Bayesian Computation (ABC) approach and

27   an equilibrium island model (right-hand column) are given. (C) Summary of patterns of genetic

28   differentiation for the 10 population pairs from Hidden Markov Models (HMMs).

(A) Pair	Locality	Species	Host 1	Host 2	n1/n2	Latitude (N)	Longitude (W)
1	BCBOG	*T. landelsensis*	*Ceanothus*	*Quercus*	19/20	36.0660	-121.5806

2	BCTURN	*T. knulli*	*Ceanothus*	*Pinus*	16/16	36.0762	-121.6064
3	BMCG3	*T. podura*	*Quercus*	*Calocedrus*	20/20	33.8313	-116.7412
4	BMT	*T. podura*	*Ceanothus*	*Quercus*	20/18	33.8273	-116.7955
5	BS	*T. chumash*	*Ceanothus*	*Quercus*	20/20	33.8164	-116.7902
6	CR	*T. sp.* 'cuesta ridge'	*Ceanothus*	*Cupressus*	20/20	35.3562	-120.6543
7	HFRS	*T. chumash*	*Ceanothus*	*Quercus*	17/18	34.3556	-118.0120
8	LP	*T. californicum / T. poppensis*	*Quercus*	*Pseudotsuga*	20/16	37.1019	-121.8756
9	SM	*T. californicum / T. poppensis*	*Quercus*	*Sequoia*	20/19	37.0188	-121.7256
10	VP	*T. cristinae*	*Ceanothus*	*Quercus*	20/20	34.5325	-119.8467

(B)	Locality	Posterior median of $N_e m$	Lower bound 90 % CI	Upper bound 90 % CI	Equilibrium estimate
1	BCBOG	1.3037	0.4112	3.1517	5.9647
2	BCTUR	0.5919	0.2450	3.0173	1.7486
3	BMCG3	1.4501	0.2338	3.7673	7.3929
4	BMT	1.8287	0.5793	4.4588	7.3281
5	BS	0.5668	0.0620	7.8986	7.4081
6	CR	1.0743	0.2888	2.7388	6.1451
7	HFRS	0.2438	0.0107	19.0472	1.5236
8	LP	0.0078	0.0021	0.0380	0.0972
9	SM	0.0067	0.0003	0.1233	0.1072
10	VP	1.6659	0.6283	3.8307	7.6160

(C)	Locality	Number of regions of accentuated F_{ST}	Mean size of regions of accentuated F_{ST} (# 20-kb windows)	Mean F_{ST} of regions of accentuated F_{ST}	Mean background F_{ST}
1	BCBOG	2	980	0.056	0.039

16

2	BCTUR	0	N/A	N/A	0.125
3	BMCG3	3	690	0.040	0.032
4	BMT	0	N/A	NA	0.033
5	BS	1	241	0.045	0.033
6	CR	2	262	0.080	0.038
7	HFRS	0	N/A	N/A	0.141
8	LP	5	408	0.789	0.714
9	SM	10	242	0.784	0.691
10	VP	0	N/A	N/A	0.032

1

2 **Supplementary Figure 1. Genetic structure and gene flow.** (A) Lack of admixture between

3 species in analyses of genetic structure (codes below species names are for locality name and

4 host). (B) Mean genetic differentiation (F_{ST}) between conspecific ecotypes and species studied

5 using whole-genome re-sequencing (these are a subset of those studied using genotyping-by-

6 sequencing data). Gene flow estimates are shown from Approximate Bayesian Computation and

7 from island-equilibrium estimates.
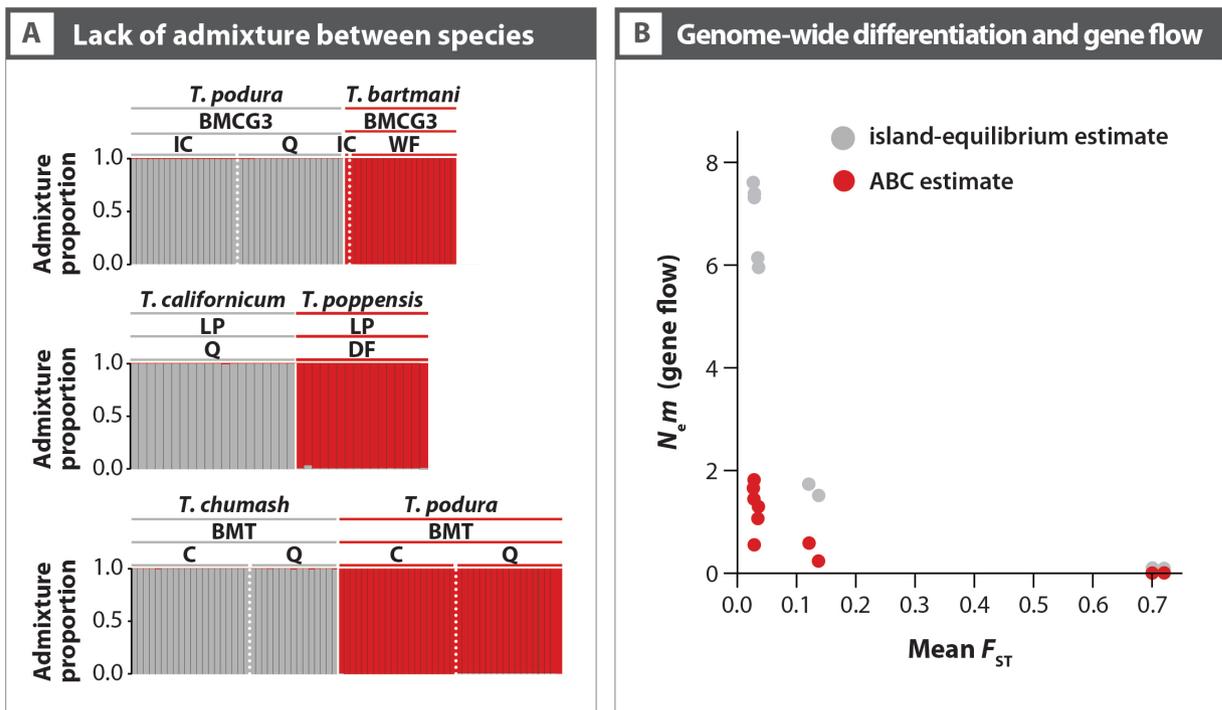
8

9 **Supplementary Table 4**. **Results of the Hidden Markov Model (HMM) analyses of genetic**

10 **differentiation between 14 pairs of _Timema_ taxa.** These are the same taxa depicted in Figure 2

1 of the main text. LG = linkage group. Numbers in parentheses following taxon pair codes

2 represent the summed number of regions of accentuated F_{ST} across LGs. Values in the body of

3 the table refer to the number of regions of accentuated differentiation per LG, followed in italics

4 by the proportion of the LG involved (i.e., for LG with at least one such region of accentuated

5 differentiation).

Taxon pair	LG1	LG2	LG3	LG4	LG5	LG6	LG7
HV (0)	0	0	0	0	0	0	0
MR1 (0)	0	0	0	0	0	0	0
R12 (3)	0	0	0	0	0	0	0
LAPRC (2)	0	1, *0.055*	0	0	0	0	0
VP (0)	0	0	0	0	0	0	0
BS (1)	0	0	1, *0.047*	0	0	0	0
BMCG3 (3)	0	0	3, *0.407*	0	0	0	0
BMT (0)	0	0	0	0	0	0	0
BCBOG (3)	0	0	0	0	0	0	0
BCTUR (0)	0	0	0	0	0	0	0
CR (2)	0	0	0	0	0	0	0
HFRS (0)	0	0	0	0	0	0	0
LP (5)	0	0	1, *0.057*	0	1, *0.107*	0	0
SM (10)	0	0	1, *0.053*	4, *0.338*	1, *0.071*	0	1, *0.084*

	LG8	LG9	LG10	LG11	LG12	LG13
HV (0)	0	0	0	0	0	0
MR1 (0)	0	0	0	0	0	0
R12 (3)	3, *0.590*	0	0	0	0	0
LAPRC (2)	1, *0.226*	0	0	0	0	0
VP (0)	0	0	0	0	0	0
BS (1)	0	0	0	0	0	0
BMCG3 (3)	0	0	0	0	0	0
BMT (0)	0	0	0	0	0	0
BCBOG (3)	2, *0.653*	1, *0.357*	0	0	0	0

BCTUR (0)	0	0	0	0	0	0
CR (2)	2, *0.247*	0	0	0	0	0
HFRS (0)	0	0	0	0	0	0
LP (6)	1, *0.117*	0	1, *0.260*	2, *0.584*	0	0
SM (10)	1, *0.022*	0	0	2, *0.415*	0	0

1

2 **Genetic differentiation and allele frequencies.** We tested whether HMM regions of

3 accentuated differentiation (F_{ST}) had MAF that differed from the genomic background (*i.e.*, for

4 the eight of 14 taxon pairs with at least one such region). The approach was as follows: for each

5 population, we inferred maximum-likelihood site MAFs from the genotype likelihoods by means

6 of the iterative soft expectation-maximization algorithm (EM), as before. We then evaluated

7 whether MAFs for the sets of HMM regions of accentuated differentiation were different from

8 MAFs of similar sets of random genomic regions. For each population (defined by the host plant,

9 "H. 1" and "H. 2" in Supplementary Table 5), we calculated an observed multi-region MAF

10 across the HMM regions of accentuated differentiation as the mean MAF of all SNPs with a

11 MAF greater than 1% within such regions ("Obs. 1" and "Obs. 2" in Supplementary Table 5, one

12 observed value for each population in the pair). We obtained the empirical null distribution of

13 MAFs for random genomic regions by means of a randomisation procedure. For each population

14 pair, we randomly sampled without replacement the same number of genomic regions of the

15 same size as the corresponding HMM regions of accentuated differentiation. Subsequently, we

16 calculated multi-region mean MAFs for each population, as before. We repeated this procedure

17 1000 times in order to obtain the null distribution of 1000 multi-region MAFs ("Null expectation

18 1" and "Null expectation 2" in Supplementary Table 5; mean and 95% confidence intervals are

19 shown). We assessed significance by computing empirical cumulative distributions and

20 calculating two-tail *P*-values ("*P* 1" and "*P* 2" in Supplementary Table 5).

21

22 We found that MAFs in the HMM regions of accentuated differentiation sometimes differed

23 from the genomic background, but not in a consistent way. When we detected differences, they

24 were weak in magnitude (on the order of ~1%) and varied in sign among taxon pairs (i.e.,

25 sometimes being higher and sometimes lower than the genomic background). Thus, we did not

26 observe a strong and consistent overall association between allele frequency and F_{ST}, as may be

1 expected when F_{ST} is estimated for larger windows containing many SNPs, rather than for

2 individual SNPs.

3

4 **Supplementary Table 5.** Minor allele frequency (MAF) of HMM regions of accentuated

5 differentiation compared to null genomic background expectations. H = host plant. NA = not

6 applicable (*i.e.*, population pairs that did not have at least one region of accentuated

7 differentiation in the HMM analyses). Obs. = empirically observed MAFs for each population in

8 a population pair). *P* 1 and *P* 2 are the significance values for each population.

Populations			Null expectation 1				Null expectation 2			
Locality	H 1	H 2	Obs. 1	mean	95% CI	*P* 1	Obs. 2	mean	95% CI	*P* 2
HV	A	C	NA	NA	NA	NA	NA	NA	NA	NA
MR1	A	C	NA	NA	NA	NA	NA	NA	NA	NA
R12	A	C	0.159	0.146	0.138-0.159	0.042	0.171	0.154	0.145-0.171	0.058
LA/PRC	A	C	0.178	0.148	0.140-0.164	0.014	0.177	0.163	0.155-0.174	0.004
BCBOG	C	Q	0.197	0.222	0.202-0.229	0.006	0.263	0.250	0.242-0.261	0.014
BCTUR	C	P	NA	NA	NA	NA	NA	NA	NA	NA
BMCG3	IC	Q	0.196	0.215	0.205-0.230	0.000	0.215	0.225	0.219-0.238	0.000
BMT	C	Q	NA	NA	NA	NA	NA	NA	NA	NA
BS	C	Q	0.155	0.172	0.144-0.203	0.280	0.218	0.230	0.202-0.267	0.498
CR	C	CY	0.225	0.223	0.212-0.236	0.682	0.224	0.225	0.214-0.236	0.928
HFRS	M	Q	NA	NA	NA	NA	NA	NA	NA	NA
LP	DF	Q	0.214	0.213	0.186-0.233	0.962	0.232	0.235	0.227-0.247	0.554
SM	Q	RW	0.184	0.208	0.186-0.251	0.010	0.223	0.230	0.223-0.237	0.058
VP	C	Q	NA	NA	NA	NA	NA	NA	NA	NA

9

10 **Quantifying colour-pattern and CHCs** We recorded digital images of 873 adult *T. cristinae*

11 using previously described methods[21] (Supplementary Table 6, Supplementary Fig. 4 for map of

12 localities).

13

14 **Supplementary Table 6.** Identity, locality, and sample sizes of populations and species used to

15 study phenotypic variation in cuticular hydrocarbons (CHCs) and colour pattern (% body area

16 striped). Note that we used some datasets in several analyses such that sample sizes are not

17 unique to just one analysis. In addition to the numbers in the table, the perfuming experiment

18 included 96 insects from the FHA population (24 males, 72 females), 24 females from the

1   SMRW population, and 24 females from the SMHCRW population. Abbreviations for host plant

2   (*A = Adenostoma, C = Ceanothus, S = Sequoia*).

Population code	Latitude (N)	Longitude (W)	Host	CHC variation *N* (males, females)	% body area striped *N* (males, females)
T. cristinae					
FHA	34.517644	-119.800989	*A*	20 (10, 10)	20 (10, 10)
ECC20A	34.504972	-119.73285	*A*	18 (10, 8)	18 (10, 8)
PC	34.476789	-119.768839	*C*	20 (10, 10)	20 (10, 10)
MH19.78C	34.519144	-119.270992	*C*	10 (5, 5)	10 (5, 5)
MH25.59C	34.533242	-119.243072	*C*	20 (10, 10)	20 (10, 10)
R12C	34.515031	-119.071031	*C*	20 (10, 10)	20 (10, 10)
R23A	34.519111	-119.077511	*A*	20 (10, 10)	-
HVA	34.488586	-119.785839	*A*	20 (10, 10)	20 (10, 10)
LA	34.512586	-119.796203	*A*	20 (10, 10)	-
PRC	34.533308	-119.857644	*C*	20 (10, 10)	20 (10, 10)
OGC	34.513442	-119.796086	*C*	15 (8, 7)	15 (8, 7)
NS1A	34.488361	-119.654611	*A*	19 (9, 10)	18 (9, 9)
MA	34.515103	-119.797133	*A*	16 (10, 6)	16 (10, 6)
ECCCampA	34.506411	-119.761644	*A*	9 (5, 4)	9 (5, 4)
OGA	34.513406	-119.796322	*A*	18 (8, 10)	17 (8, 9)
ECC35A	34.5062	-119.768136	*A*	18 (10, 8)	18 (10, 8)
OUTA	34.531683	-119.843517	*A*	16 (9, 7)	16 (9, 7)
BYA	34.5006	-119.86195	*A*	20 (10, 10)	20 (10, 10)
SC	34.5226	-119.83175	*C*	19 (10, 9)	19 (10, 9)
MH29.19C	34.555367	-119.263167	*C*	5 (0, 5)	5 (0, 5)
T. poppensis					
SMRW	37.01876	-121.72556	*S*	-	-
SMHCRW	37.01074	-121.71508	*S*	-	-

3

4   For CHC variation, we sampled 20 different populations of *Timema cristinae* (eight on

5   *Ceanothus* and 12 on *Adenostoma*) for a total of 915 individuals (559 males and 356 females),

6   and two populations of *Timema poppensis* (48 females). To extract CHCs from the body surface

1   of individual insects, we euthanized insects by 1-h freezing, and then submerged each insect for

2   10 minutes in 1 ml of HPLC-grade hexane in separate vials. Subsequently, we removed the

3   insect from each vial, concentrated the sample to dryness by hexane evaporation at room

4   temperature, and re-constituted the CHC extract by adding 100 µl of hexane containing (E)-9-

5   octadecenyl acetate as an internal standard (IS). We then analysed an aliquot of each sample on a

6   6890 Hewlett Packard (now Agilent) gas chromatograph (GC) equipped with a DB-5 MS

7   column (50 m × 0.25 mm i.d.), using the following temperature program: 100 °C for 1 min, then

8   20 °C per min to 280 °C. The final temperature of 280 °C was held for 40 min. Temperatures of

9   the GC injector and the flame ionization detector (FID) were set to 280 °C.

10

11 In total, we quantified 26 different mono- and di-methylated CHCs for each individual *T.*

12 *cristinae*. Specifically, we quantified eight methylated pentacosanes, eight methylated

13 heptacosanes (including the six monomethyl heptacosanes previously described[22]), and 10

14 methylated nonacosanes. As is standard practice in studies of CHC variation[22], we analysed

15 proportional rather than absolute abundances of CHCs; this allowed us to reduce experimental

16 error and to remove individual differences in CHCs stemming from insect body size variation

17 [23,24]. We determined the total amount of each target CHC by multiplying the area count of the

18 respective FID peak with 200 ng of the IS and by dividing the product by the FID area count of

19 the IS. We calculated proportional CHCs by dividing the amount of each CHC in a given sample

20 by the sum of all CHCs in that sample. We then transformed these CHC proportions using log-

21 contrasts[23,25] to remove the non-independence among analysed variables. We calculated log-

22 contrasts by dividing the value for each CHC by the value of the CHC 5-methylheptacosane

23 (5Me27), and then taking the $\log_{10}$ of these new variables, resulting in 25 log-contrast

24 transformed values for every insect. We obtained similar results when we divided the value for

25 each CHC by the value of a CHC other than 5Me27.

26

27 **Repeatability of CHC measurements.** To test the repeatability of our phenotypic measures (i.e.,

28 proportional CHCs), we randomly chose hexane extracts of six males and six females each from

29 two different populations (FHA and MH25.59C), and analysed them once each on two

30 consecutive days, using the protocols described above. We again calculated log-contrasts for

31 proportions of all 25 CHCs (contrasting against the 26th CHC, 5Me27) and calculated intra-class

1 correlation coefficients (ICC, $n = 24$)[26] in IBM SPSS Statistics 21 (IBM Corporation). ICC

2 analyses revealed very high repeatability for every single CHC (ICC, $r \geq 0.859$ in all cases, as

3 follows for each compound: LogC_C25-1, 0.964; LogC_C25-2, 0.891; LogC_C25-3, 0.972;

4 LogC_C25-4, 0.998; LogC_C25-5, 0.989; LogC_C25-6, 0.973; LogC_C25-7, 0.976;

5 LogC_C25-8, 0.933; LogC_C27-1, 0.986; LogC_C27-2, 0.947; LogC_C27-4, 0.955;

6 LogC_C27-5, 0.859; LogC_C27-6, 0.933; LogC_C27-7, 0.987; LogC_C27-8, 0.968;

7 LogC_C29-1, 0.929; LogC_C29-2, 0.952; LogC_C29-3, 0.940; LogC_C29-4, 0.998;

8 LogC_C29-5, 0.965; LogC_C29-6, 0.950; LogC_C29-7, 0.931; LogC_C29-8, 0.874;

9 LogC_C29-9, 0.980; LogC_C29-10, 0.919).

10

11 **Differences between ecotypes in CHCs.** We conducted this analysis on CHC extracts of 343 *T.*

12 *cristinae* from 20 different populations sampled in 2013 (174 males and 169 females from 12

13 populations adapted to *Adenostoma* and eight populations adapted to *Ceanothus*). Because we

14 measured many more individuals in the FHA population than in other populations to accomplish

15 GWA mapping, we randomly chose 10 males and 10 females from FHA for this analysis. Across

16 all populations, we detected five samples that were extreme multivariate CHC outliers based on

17 Mahalanobis distance as calculated in the SPSS 'Regression' procedure, and we thus removed

18 them from subsequent analyses (i.e., one male from OGC, one female each from ECC35A and

19 ECCCampA, and two females from ECC20A).

20

21 To reduce data dimensionality and to account for multicollinearity, we conducted a principal

22 components analysis (on a covariance matrix with promax rotation) on the remaining 338

23 samples (172 males and 166 females). We retained principal component (PC) axes with an

24 eigenvalue larger than the mean eigenvalue as variables for subsequent analyses (resulting in six

25 axes retained, which accounted for 89.5% of the total variation; Supplementary Table 7). We

26 then conducted multivariate analysis of variance (MANOVA) on these six PCs as our primary

27 test of phenotypic differences between sexes and ecotypes, by testing for effects due to 'sex',

28 'host plant', and the interaction of 'sex-by-host plant'.

29

1   **Supplementary Table 7.** Loadings for principal components (PC) analyses carried out on

2   cuticular hydrocarbon (CHC) profiles of male and female *T. cristinae* from 20 different

3   populations (host plants: *Adenostoma, N* = 12; *Ceanothus, N* = 8).

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalues	13.478	4.579	3.791	2.091	1.955	1.246
% Variance	44.555	15.102	12.503	6.897	6.449	4.110
CHC compound						
LogC_C25-1	-0.001	0.719	-0.083	0.091	0.059	0.017
LogC_C25-2	0.009	1.038	0.022	-0.197	-0.024	0.068
LogC_C25-3	-0.044	0.580	0.016	0.027	0.072	-0.066
LogC_C25-4	0.041	0.101	0.070	0.915	0.041	0.018
LogC_C25-5	-0.142	0.103	-0.097	0.042	0.925	0.049
LogC_C25-6	0.188	0.617	0.060	0.129	-0.118	-0.091
LogC_C25-7	0.000	0.731	-0.012	-0.041	0.156	0.040
LogC_C25-8	-0.237	0.132	0.945	0.043	-0.079	0.144
LogC_C27-1	0.388	0.491	-0.041	-0.008	0.050	-0.108
LogC_C27-2	0.187	0.504	-0.025	0.126	-0.007	-0.088
LogC_C27-4	0.524	0.297	0.064	0.081	-0.082	0.143
LogC_C27-5	0.509	0.218	0.003	0.041	-0.030	0.079
LogC_C27-6	-0.073	0.281	0.140	0.025	0.028	-0.115
LogC_C27-7	0.550	0.183	-0.115	0.010	-0.011	-0.073
LogC_C27-8	-0.041	0.221	0.329	-0.034	0.000	-0.110
LogC_C29-1	0.775	-0.067	-0.072	-0.068	0.009	0.016
LogC_C29-2	0.580	-0.002	-0.227	-0.046	-0.036	0.076
LogC_C29-3	0.575	0.076	-0.163	0.016	-0.062	0.081
LogC_C29-4	0.235	-0.177	-0.001	0.017	0.051	0.853
LogC_C29-5	0.703	-0.081	-0.083	0.044	0.002	0.013
LogC_C29-6	0.666	0.019	-0.065	0.042	-0.064	0.021
LogC_C29-7	0.094	0.176	-0.264	-0.048	0.034	0.086
LogC_C29-8	-0.151	0.466	-0.022	-0.037	-0.089	0.008
LogC_C29-9	0.551	-0.130	-0.122	-0.016	-0.018	0.025

LogC_C29-10	0.867	-0.197	0.479	-0.083	0.126	-0.086

1

2 **Genomic data from population FHA for GWA mapping.** We obtained genotypes for mapping

3 from publicly available sequence data for 602 *T. cristinae* individuals from the FHA population

4 (NCBI BioProject PRJNA284835)[21]. From the 524,832 SNPs obtained in that previous study, we

5 created subsets for the 592 individuals for which CHC data were available, as well as for males

6 and females separately. We discarded variants with a MAF below 1% in each subset. The

7 resulting datasets comprised 246,258 variants (all 592 individuals), 246,293 variants for females

8 (197 individuals), and 245,778 for males (395 individuals). As in past work[1], we used a custom

9 Perl script to calculate empirical Bayesian posterior probabilities for the genotypes of each

10 individual and locus using the genotype likelihoods and allele frequencies estimated by

11 BCFTOOLS along with Hardy-Weinberg priors (i.e. $p(AA) = p_i^2$; $p(aa) = (1-p_i)^2$; $p(Aa) = 2p_i(1-p_i)$;

12 'A' is the major allele, 'a' is the minor allele, and 'p' is the major allele frequency). Finally, we

13 calculated the posterior mean genotype for each individual, at each locus, defined as the minor

14 allele dosage (i.e., *g-hat*$_{ij}$ as $\Sigma_{k = \{0, 1, 2\}} k * Pr(g_{ij} = k|$ data, $p_i)$, where g_{ij} is the genotype for locus i

15 and individual j, and k are the variants). We used these imputed genotypes for all GWA mapping

16 analyses.

17

18 **Genome-wide association (GWA) mapping and cross-validation.** We used the software

19 GEMMA 0.94 for GWA mapping[27]. We used GEMMA to implement Bayesian sparse linear mixed

20 models (BSLMMs) using a multiple-SNP Bayesian approach to model the genetic architecture of

21 traits while considering relatedness of individuals. In BSLMMs implemented in GEMMA the

22 effects of SNPs are modelled as coming from a mixture of two normal distributions. Thus,

23 effects of SNPs that individually have infinitesimal effects ('polygenic distribution') and SNPs

24 with measurable (i.e., 'larger' or 'sparse') effects can be estimated. GEMMA also provides

25 posterior inclusion probabilities (PIPs, also called γ parameter) that reflect the weight of

26 evidence that individual SNPs are associated with the trait of interest.

27

28 We estimated the above-mentioned hyper-parameters and PIP values for the following seven

29 traits: % striped, the proportion of methylated pentacosanes, heptacosanes, and nonacosanes in

30 females (fpenta, fhepta, and fnona, respectively), and the proportion of methylated pentacosanes,

1   heptacosanes, and nonacosanes in males (mpenta, mhepta, and mnona, respectively). We treated

2   sexes separately for CHCs due to strong sexual dimorphism in CHCs. For % striped, we ran

3   GEMMA on residuals that corrected for differences between sexes by regressing each trait against

4   sex. We report for each trait the point estimates (median) and 95% equal-tail probability intervals

5   (ETPIs) of hyper-parameters, calculated across 10 independent MCMC runs per trait. For each

6   chain, we ran 20,000,000 iterations with a recording pace of one record state in every 100 steps

7   and discarded the first 5,000,000 iterations as burn-in. We excluded SNPs with a MAF less than,

8   or equal to, one percent.

9

10   Following these standard GWA runs, we performed cross-validation analyses to test the

11   predictive power of our GWA. The approach is akin to that commonly taken in genomic

12   prediction/genomic selection studies[28]. For each trait, we estimated a predicted phenotype (based

13   on genotype) for each individual by randomly masking 10% of individual phenotypes ('test set')

14   [27] and using the remaining 90% of phenotypes ('training set') to obtain model parameters in

15   GEMMA using the same parameters as in the standard runs. We then used these parameters in

16   GEMMA to obtain predicted phenotypic values using the '–predict' option[27]. In each instance, we

17   ran 10 replicate MCMC chains for each training set and repeated this procedure 10 times (i.e.,

18   until we had obtained predicted values for every individual). We repeated the entire process 10

19   times with different random combinations of individuals in each training set to avoid any

20   potential 'training set' biases, resulting in a total of 100 predicted phenotypes for each observed

21   phenotype.

22

23   We then estimated the reliability of genomic prediction by correlating the mean predicted

24   phenotypic values against the observed individual phenotypic values. For CHCs, GEMMA

25   predicted values were logit-transformed because the CHC phenotypes are proportional data[29];

26   this transformation provides a more conservative estimate of the correlation. We report the

27   square of the correlation coefficient (r) and its significance. This r-squared value estimates the

28   phenotypic variation due to estimated additive genetic effects, with an upper limit being the ratio

29   of observed genetic variance (VG) to phenotypic variance[28], which is reported by GEMMA as
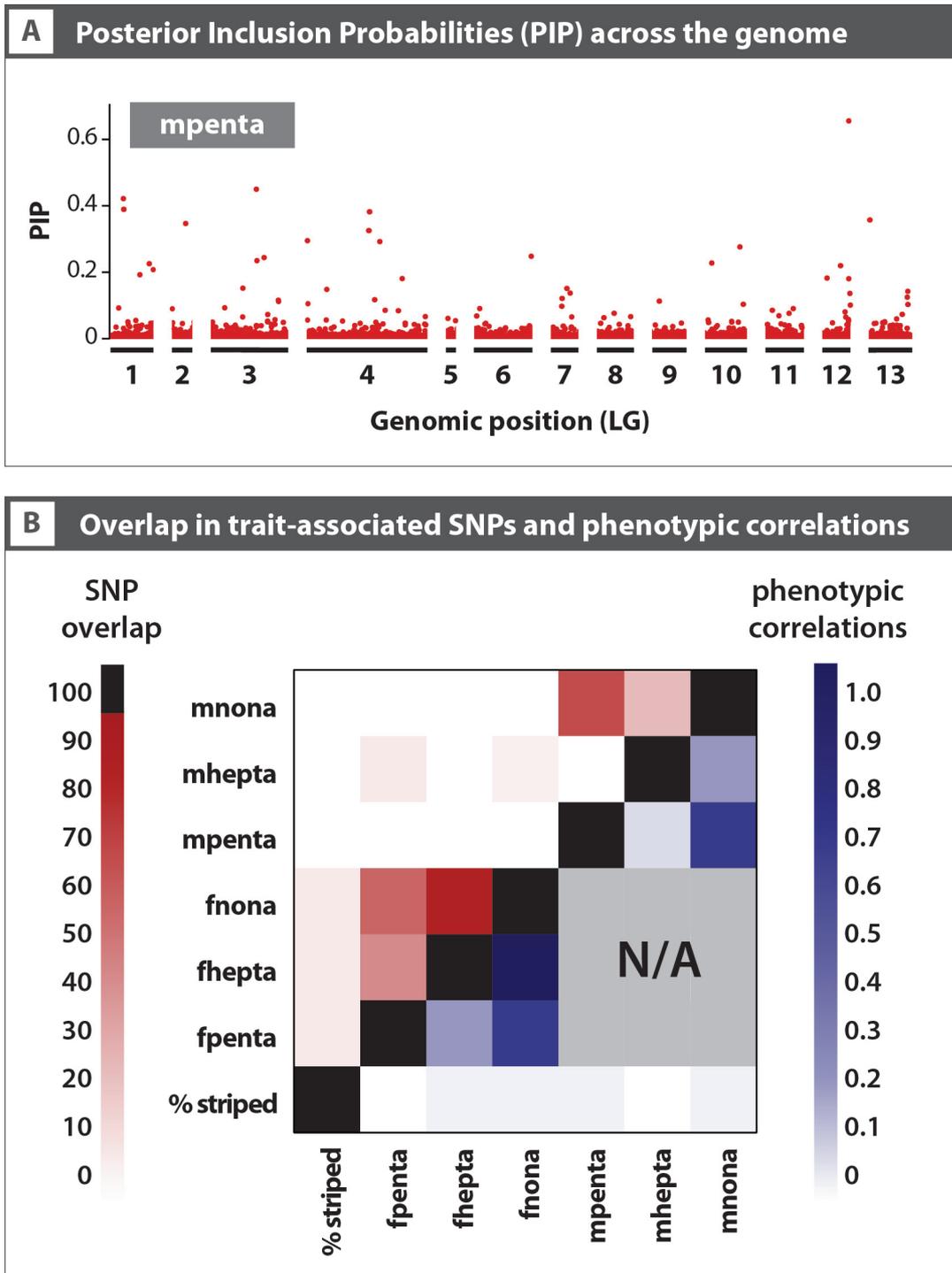
30   PVE.

1 **Supplementary Table 8**. **Genetic architecture of the seven traits studied.** PVE: proportion of

2 phenotypic variance explained by genetic data (all SNPs); PGE: proportion of genetically

3 explained phenotypic variance due to sparse (measurable) genetic effects; n-SNP: number of

4 SNPs with measurable effect. CHCs = cuticular hydrocarbons. Given are median values and 95%

5 equal-tail probability intervals (ETPIs). Values of r^2 are predictive power from cross-validation

6 runs.

Genetic architecture of non-CHC traits				Genetic architecture of methylated CHCs			
parameter	median	2.5% bound	97.5% bound	parameter	median	2.5% bound	97.5% bound
1) % body area striped (% striped)				2) proportion of female pentacosanes (fpenta)			
PVE	0.68	0.62	0.77	PVE	0.43	0.04	0.76
PGE	0.97	0.88	1.00	PGE	0.22	0.00	0.92
n-SNP	12	6	28	n-SNP	17	0	259
$r^2 = 0.582, P < 0.001$				$r^2 = 0.010, P = 0.153$			
3) proportion of female heptacosanes (fhepta)				4) proportion of female nonacosanes (fnona)			
PVE	0.27	0.01	0.68	PVE	0.32	0.02	0.71
PGE	0.29	0.00	0.95	PGE	0.26	0.00	0.94
n-SNP	29	0	275	n-SNP	30	0	276
$r^2 = 0.040, P = 0.005$				$r^2 = 0.032, P = 0.012$			
5) proportion of male pentacosanes (mpenta)				6) proportion of male heptacosanes (mhepta)			
PVE	0.74	0.17	1.00	PVE	0.64	0.12	1.00
PGE	0.15	0.00	0.83	PGE	0.16	0.00	0.80
n-SNP	51	0	281	n-SNP	17	0	266
$r^2 = 0.021, P = 0.003$				$r^2 = 0.016, P = 0.012$			
7) proportion of male nonacosanes (mnona)							
PVE	0.70	0.21	1.00				
PGE	0.29	0.00	0.93				

n-SNP   47   0   277

$r^2 = 0.042$, $P < 0.001$

1

2 **Supplementary Figure 2. Details of genome-wide association mapping.** (A) Posterior

3 inclusion probabilities (PIP) of SNPs along the genome for a representative trait (mpenta = male

4 methylated pentacosanes). Higher PIP values are indicative of stronger association with

5 phenotypic variation. (B) Heat map showing the number of the 100 most strongly trait-associated

6 SNPs per trait shared between different traits (above diagonal) and phenotypic associations

7 between traits (r^2 values; traits were transformed as for GWA). N/A = Not applicable. % striped

8 = percent body area striped, fpenta = female methylated pentacosanes, fhepta = female

9 methylated heptacosanes, fnona = female methylated nonacosanes, mpenta = male methylated

10 pentacosanes, mhepta = male methylated heptacosanes, mnona = male methylated nonacosanes.

A Posterior Inclusion Probabilities (PIP) across the genome

B Overlap in trait-associated SNPs and phenotypic correlations

1

Linkage group partitioning analysis. A prediction for polygenic traits is that the number of

trait-associated SNPs per LG will be positively correlated with the size of a LG[30]. We tested and

largely supported this prediction, as reported in the main text. We calculated a point estimate for

the number of trait-associated SNPs per LG by summing the PIPs across all SNPs on the LG, and

1 defined LG size as the number of SNPs in the GWA analysis for that LG for each trait. For

2 individual traits, number of trait-associated SNPs per LG was significantly, positively correlated

3 with LG size for all six CHC traits (fpenta, r = 1.00; fhepta, r = 1.00; fnona, r = 1.00; mpenta, r =

4 1.00; mhepta, r = 1.00; mnona, r = 1.00, all *P* < 0.05), but not for % striped (r = 0.36, *P* = 0.22).

5

6 **Perfuming trials with no-choice mating experiments.** We conducted perfuming experiments

7 to test if CHCs are important in *T. cristinae* mate choice. We collected juvenile *T. cristinae* from

8 one study site (FHA) in the Santa Ynez Mountains, California, USA, between March and April

9 2014, and juvenile *T. poppensis* in late April and early May 2014 from two study sites (SMRW

10 and SMHCRW) in the Santa Cruz Mountains, California, USA, where they feed on coastal

11 redwood, *Sequoia sempervirens*. We captured insects as early instars using sweep nets and

12 reared them to maturity in separate-sex containers in the laboratory on the foliage of their native

13 host plant collected at the site of population origin (*A. fasciculatum* for *T. cristinae* and *S.*

14 *sempervirens* for *T. poppensis*). For the no-choice copulation trials, we randomly selected

15 individual *T. cristinae* from the laboratory population, tested them once, and then euthanized

16 them. Protocols for the no-choice copulation trials used in this study are based on previously

17 published protocols[31], but were slightly altered to gain information not only on whether

18 copulation occurred, but also when it occurred. We confined one male and one female *T.*

19 *cristinae* in a 10-cm Petri dish for 4 h. For the first 15 min, we took an 'all occurrence

20 continuous sampling' approach and during the remaining 225 min, we conducted 'scan

21 sampling' at 15-min intervals to record for each interval if copulation occurred[32]. Based on

22 *Timema* mating behaviour[33], we specifically scored whether or not a pair was copulating (male

23 sits on the female's back with their genitals interlocked).

24

25 Each individual perfume consisted of CHCs extracted and pooled from six adult females. We

26 created 'conspecific' native population CHC perfumes using hexane-extracted CHCs from six

27 randomly selected virgin females from the same population (FHA), approximately 24 h after

28 females had molted into sexually mature adults modified from [34,35]. We created 'heterospecific'

29 CHC perfumes using hexane-extracted CHCs from six randomly selected virgin females of *T.*

30 *poppensis,* again approximately 24 h after they had molted into mature adults. To make a

31 perfume, we euthanized six live females by 1-h freezing, and submerged them, one female at a

1   time, in the same 1 ml of HPLC-grade hexane for 10 min to extract the CHCs from their body

2   surface. We removed each female before adding the next.

3

4   We let the hexane extract passively evaporate to dryness at room temperature, inserted a live trial

5   female into the vial containing the residual CHCs of the six extracted females, and gently hand-

6   vortexed the vial for 1 min to facilitate CHC transfer from the vial's walls to the body surface of

7   the trial female. We applied the same procedure for females of the control (no perfume)

8   treatment, except that we hand-vortexed these females in clean vials. We allowed all trial

9   females to recover for 10 min from the perfuming procedure before the onset of a mating trial.

10

11   In total, we conducted 24 no-choice copulation trials (eight trials each with 'conspecific native

12   population perfume', 'heterospecific perfume', and 'no perfume') between one male and one

13   female *T. cristinae* from the FHA population. We conducted perfuming trials during the same

14   time of day (8:45 am – 12:45 pm) on different days, and on each day ran the same number of

15   'conspecific' and 'heterospecific' perfuming trials simultaneously. We conducted all 'no

16   perfume' trials during the last two days of testing. We analysed the latency to copulate (i.e.,

17   minutes until copulation) by means of a Kaplan-Meyer analysis in IBM SPSS Statistics 21 (IBM

18   Corporation). Our perfuming protocol led to strong effects on mate choice (Supplementary Table

19   9), which is congruent with previous studies in other insect systems[34-36].

20

21   **Supplementary Table 9. Treatment comparisons from the perfuming experiment.** All-

22   pairwise comparisons (Log Rank tests) of the three treatments ('conspecific perfume',

23   'heterospecific perfume', and 'no perfume') for the no-choice copulation trials between one male

24   and one female *T. cristinae*. Significant results are in bold.

Treatment	Conspecific perfume		Heterospecific perfume		No perfume	
	Chi^2	P	Chi^2	P	Chi^2	P
Conspecific perfume			16.512	**<0.001**	8.364	**0.004**
Heterospecific perfume	16.512	**<0.001**			14.681	**<0.001**
No perfume	8.364	**0.004**	14.681	**<0.001**		

25

1 **Morph frequency cline in *T. cristinae.*** Sampling and analytical details are contained in the

2 methods section and a map of localities is available in Supplementary Fig. 4. Full data and

3 locality information is provided in Supplementary Table 10 and results in the Supplementary Fig.

4 3 below.

5

6 **Supplementary Table 10. Sample sites and morph frequencies for the cline analysis.** G =

7 green-unstriped, S = green-striped, I = intermediate, M = melanistic. C = *Ceanothus*. A =

8 *Adenostoma.* Zero values for a locality across all morphs are true zeros, not due to lack of

9 sampling the locality (N/A = not applicable due to no individuals being collected).

Locality number	2001				1996				Host	Latitude (N)	Longitude (W)
	G	S	I	M	G	S	I	M			
1	0	0	0	0	4	0	2	1	C	34.493	-120.066
2	7	0	0	1	2	0	2	0	C	34.508	-120.065
3	0	0	0	0	23	0	4	2	C	34.509	-120.065
4	2	0	0	0	20	1	16	2	C	34.510	-120.069
5	4	0	0	0	17	1	4	5	C	34.512	-120.069
6	0	0	0	0	3	0	2	0	C	34.513	-120.072
7	3	1	0	1	6	4	9	2	C	34.513	-120.074
8	3	1	1	0	12	10	8	6	C	34.514	-120.075
9	4	2	0	1	1	1	2	2	C	34.514	-120.072
10	0	0	0	0	4	3	1	2	C	34.515	-120.071
11	0	0	0	0	3	4	2	1	A	34.515	-120.071
12	17	15	2	2	88	50	50	33	C	34.515	-120.071
13	1	10	1	0	74	111	73	13	A	34.515	-120.071
14	0	0	0	0	16	4	3	7	C	34.515	-120.072
15	4	1	0	0	24	5	1	4	C	34.516	-120.073
16	6	1	4	1	23	10	44	14	C	34.516	-120.073
17	6	1	0	2	10	3	16	5	C	34.517	-120.074
18	10	5	2	0	2	1	5	0	C	34.517	-120.074
19	14	6	2	1	9	9	15	7	C	34.517	-120.075
20	0	0	0	0	0	2	0	0	C	34.517	-120.075
21	1	7	1	0	4	55	19	8	A	34.517	-120.075
22	0	8	1	0	0	36	2	1	A	34.517	-120.076
23	0	0	0	0	0	0	0	0	C	N/A	N/A

24	0	0	0	0	0	0	0	0	A	N/A	N/A
25	0	0	0	0	1	2	3	4	C	34.517	-120.076
26	4	91	7	1	2	25	9	5	A	34.518	-120.077
27	0	6	3	1	4	19	12	1	A	34.529	-120.073
28	0	0	0	0	11	11	11	4	C	34.529	-120.074
29	0	1	0	0	0	0	0	0	C	34.529	-120.075
30	2	1	0	0	8	44	9	9	A	34.529	-120.075
31	0	0	0	0	11	32	17	6	A	34.530	-120.080
32	0	0	2	1	0	0	0	0	C	34.530	-120.083
33	1	3	1	0	0	0	0	0	A	34.530	-120.083

1

2

3 **Supplementary Figure 3. Cline in allele frequency, inferred from morph frequencies (grey**

4 **shaded areas are ± 95% credible intervals).** (A) Excluding intermediate phenotypes. (B)

5 Treating intermediates as green-striped morphs. (C) Treating intermediates as green-unstriped

6 morphs.
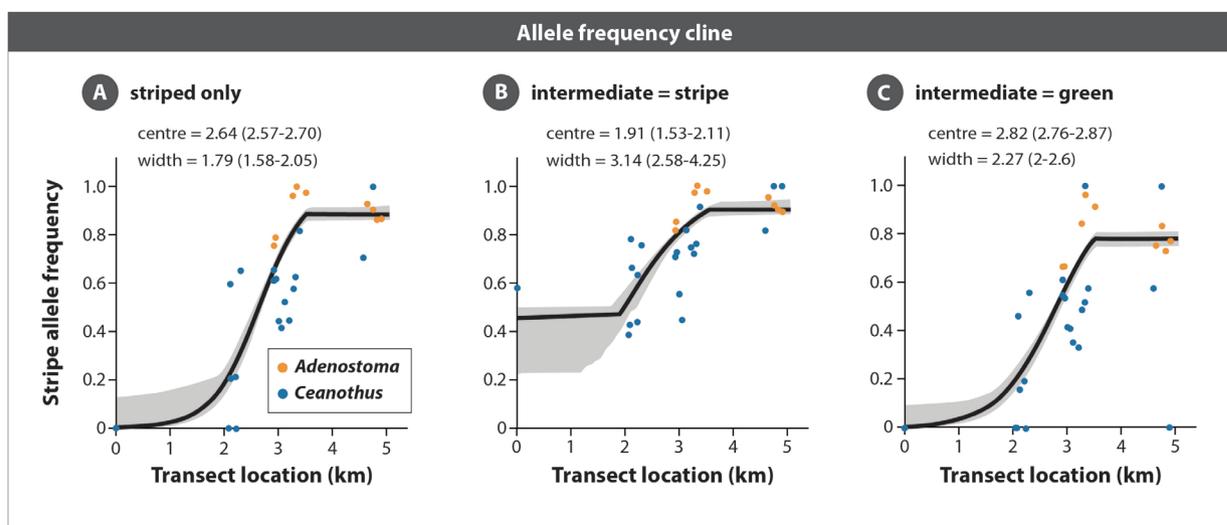
7

8 **Within-generation transplant experiment.** As the procedures for implementing this

9 experiment have been previously described[37], we provide here only a brief overview. We

10 induced host shifts in nature. To do this, we collected individual *T. cristinae* (n = 500) from

11 *Adenostoma* in an area dominated by *Adenostoma*, but in which some *Ceanothus* also occurs

12 (population FHA). We collected individuals on 14 April 2011 and placed them in 500-mL plastic

13 containers at a density of 50 individuals per container. The following day (15 April 2011), we

1   randomly assigned individuals to one of 10 experimental bushes (five of each host species). Each

2   individual then had a portion of one leg removed as a tissue sample using sterile scissors (no

3   effect of tissue sampling on survival was seen in either lab or field experiments)[37]. We moved

4   each group of 50 individuals onto either an individual of their native host plant (*Adenostoma*) or

5   the alternative host plant (*Ceanothus*) on 16 April 2011. We recaptured surviving experimental

6   insects using sweep nets and visual surveys during 24 and 25 April 2011, and took a second

7   tissue sample from these insects (n = 140). Past mark recapture work and surveys conducted in

8   this specific experiment have shown that this protocol is highly effective at recapturing the

9   overwhelming majority of surviving individuals and that dispersal across 'bare ground' (grassy

10   regions not containing suitable hosts) is near absent[37]. Thus, mortality resulted in the recaptured

11   individuals in each population at the end of the experiment being a subset of those initially

12   released (range of surviving individuals across experimental bushes = 7 – 23).

13

14   **Whole-genome re-sequencing of *T. cristinae* from the FHA population.** We re-sequenced the

15   genomes of 473 of the 500 individuals from the FHA population using previously published

16   protocols to extract DNA, to prepare individually-barcoded sequencing libraries, and to conduct

17   whole-genome re-sequencing[1] (we could not obtain data for 27 individuals which were

18   distributed across blocks and treatments). We aligned the paired-end sequences to the *T.*

19   *cristinae* reference genome (v0.3) using the BWA-MEM algorithm in BWA 0.7.5a-r405[2]. We used

20   a minimum seed length of 20 bp, set -r to 1.3 to look for internal seeds in seeds longer than 1.3 *

21   20-bp seeds, discarded chains if the seeded bases were shorter than 100 bp, and set the minimum

22   score to output an alignment to 30. We then used SAMTOOLS to compress, sort, and index the

23   alignments and to remove potential PCR duplicates. We then identified variant nucleotides using

24   the UnifiedGenotyper in GATK with the prior probability of heterozygosity set to 0.001, a

25   minimum base quality score of 20, a call confidence threshold of 50, and a maximum of 2 alleles

26   allowed. In subsequent analyses, we considered only SNPs that mapped to one of the 13

27   identified LGs (*i.e.*, due to our interest in genetic architecture, we ignored the scaffolds not

28   assigned to a LG). We further filtered the initial set of variants by retaining only those with (*i*) a

29   minimum total sequencing depth of 500, (*ii*) a minimum of 10 reads supporting the non-

30   reference allele, (*iii*) no more than 1 % of reads spanning an insertion-deletion, (*iv*) no more than

31   5 mapping quality 0 reads, (*v*) a maximum absolute value of the base quality rank sum test of 3,

1   (*vi*) a maximum absolute value of the mapping quality rank sum test of 2, (*vii*) a maximum

2   absolute value of the read position rank sum test of 2, and (*viii*) a minimum ratio of the variant

3   confidence score to the non-reference read depth of 2. We then discarded SNPs with MAF less

4   than 1%, which left us with 8.15 million SNPs for subsequent analyses.

5

6   We used an empirical Bayesian approach to estimate genotypes for the called SNPs. In particular,

7   we calculated the posterior probability of $g_{ij} = 0$, 1, or 2 non-reference alleles as $\Pr(g_{ij} \mid \text{data}, p_i) =$

8   $(\Pr(\text{data} \mid g_{ij}) \Pr(g_{ij} \mid p_i))/\Pr(\text{data})$, where i and j index a locus and individual, $\Pr(\text{data} \mid g_{ij})$ is the

9   genotype likelihood calculated with GATK's UnifiedGenotyper, and $\Pr(g_{ij} \mid p_i)$ is the probability

10 of the genotype given Hardy-Weinberg expectations and the maximum likelihood allele

11 frequency estimate from GATK. We then calculated the mean of the posterior (i.e., $g\text{-}hat_{ij}$) as $\Sigma_{k=}$

12 $_{\{0, 1, 2\}}$ $k * \Pr(g_{ij} = k \mid \text{data}, p_i)$. Finally, we obtained maximum likelihood estimates of the

13 treatment-specific allele frequencies from the genotype estimates.

14

15 **Estimation of morphological differentiation within and between species.** Methods are

16 described in the main text, and full results tabulated here in Supplementary Tables 11 and 12 .

17

18 **Supplementary Table 11.** Species, locality, and sample sizes of populations used for studying

19 phenotypic distances between populations and species. Abbreviations for the host plants (A =

20 *Adenostoma fasciculatum*, AC = *Acer macrophyllum*, C = *Ceanothus spinosus*, CY = *Cupressus*

21 *sargentii*, DF = *Pseudotsuga menziesii*, M = *Arctostaphylos* sp., MM = *Cercocarpus betuloides*,

22 P = *Pinus* sp., Q = *Quercus* sp., RW = *Sequoia sempervirens*).

Population Locality Code	Latitude (N)	Longitude (W)	Host	N total (males, females)
T. boharti				
SRHWY	32.8223	-116.505	*C*	2 (1, 1)
T. californicum				
Lick	37.3424	-121.648	*Q*	30 (15, 15)
LP	37.10186	-121.876	*AC, M, Q*	38 (18, 20)

SM	37.01876	-121.726	*M, P, Q*	16 (3, 13)
T. chumash				
BALD	34.22108	-117.668	*C, Q*	50 (24, 26)
BS	33.81641	-116.79	*C*	7 (6, 1)
GR10.43	34.22505	-117.68	*Q*	48 (25, 23)
GR8.06	34.22046	-117.707	*MM, Q*	80 (40, 40)
HF4	34.26536	-118.098	*C*	4 (2, 2)
HF6	34.26695	-118.117	*Q*	4 (2, 2)
HFDPD	34.3406	-118.016	*M, Q*	40 (21, 19)
HFRB	34.25807	-118.105	*M, Q*	3 (1, 2)
HFRS	34.35558	-118.012	*MM, Q*	35 (18, 17)
HFTP	34.34355	-117.983	*C*	32 (15, 17)
T. cristinae				
FH	34.51764	-119.801	*A*	40 (20, 20)
FIGMT	34.72803	-119.951	*Q*	4 (1, 3)
HV	34.48859	-119.786	*A, C*	25 (15, 10)
L	34.51258	-119.796	*A*	31 (20, 11)
M	34.51511	-119.797	*M*	1 (0, 1)
NH	34.51554	-119.797	*A*	18 (13, 5)
PR	34.53331	-119.858	*C*	41 (20, 21)
R12	34.51503	-120.071	*A, C*	12 (5, 7)
SC	34.5226	-119.832	*C*	23 (14, 9)
T. sp. 'cuesta ridge'				
CRH	35.36192	-120.658	*C, CY*	42 (21, 21)
CRL	35.35064	-120.647	*A, C, M, MM*	51 (25, 26)
T. knulli				
BCE	36.0713	-121.599	*RW*	36 (21, 15)
BCTUR	36.06215	-121.562	*C*	3 (1, 2)
BCXRD	36.0706	-121.591	*C*	14 (4, 10)

HB	36.16438	-121.675	*C*	9 (6, 3)
T. landelsensis				
BCBOG	36.06599	-121.581	*Q*	30 (17, 13)
BCHC	36.06266	-121.573	*M, Q*	21 (10, 11)
BCHR	36.06225	-121.565	*Q*	12 (6, 6)
BCOG	36.06266	-121.573	*C, Q*	20 (9, 11)
BCSUM	36.06544	-121.578	*C, Q*	14 (7, 7)
T. petita				
101SS	35.73057	-121.314	*C*	33 (20, 13)
T. podura				
BMTB	33.82714	-116.781	*Q*	6 (0, 6)
BS	33.81641	-116.79	*C*	4 (2, 2)
DZ243	33.85644	-116.835	*A*	10 (3, 7)
T. poppensis				
LP	37.10186	-121.876	*DF, RW*	9 (3, 6)
SM	37.01876	-121.726	*RW*	40 (20, 20)
SMHC	37.01002	-121.714	*RW*	40 (20, 20)

1

2 **Supplementary Table 12**. Trait loadings in principal components (PC) analyses. I-IV are the

3 first four PCs. Abbreviations for the traits: BL = body length, BW = body width, HW= head

4 width, latRG = lateral red-green colour channel, latGB = lateral green-blue colour channel, latL

5 = lateral luminance, dorRG = dorsal red-green colour channel, dorGB = dorsal green-blue colour

6 channel, dorL = dorsal luminance.

	Females				Males			
Variable	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
BL	0.040	0.535	-0.292	-0.007	0.049	-0.523	0.390	-0.047
BW	0.270	0.475	0.143	-0.018	0.381	-0.406	-0.102	-0.200
HW	0.237	0.563	-0.048	0.051	0.323	-0.488	-0.067	-0.106

latRG	0.397	-0.185	-0.400	0.440	0.401	0.124	0.228	0.571
latGB	-0.444	-0.014	0.153	0.268	-0.371	-0.084	-0.401	0.188
latL	0.303	0.050	0.556	0.415	0.222	-0.195	-0.632	0.096
dorRG	0.397	-0.290	-0.308	0.273	0.436	0.188	0.011	0.451
dorGB	-0.375	0.160	0.075	0.688	-0.316	-0.342	-0.230	0.541
dorL	0.353	-0.156	0.547	-0.110	0.332	0.332	-0.410	-0.283
Proportion variance	34.28%	24.34%	13.76%	11.01%	35.47%	25.54%	14.98%	11.25%

1

2 **Phylogenetic inference and molecular dating.** As with the maximum-likelihood inference of

3 bootstrap trees, we used a custom Perl script to generate a multiple alignment of 19,556 SNVs,

4 but this time we produced consensus sequences with IUPAC ambiguities by pooling the

5 individuals of every species and localities sampled (47 geographic populations in total). Mean

6 coverage per variant per locality was ~133×. As before, we partitioned the alignment by linkage

7 group (LG) and excluded the positions in genomic regions not assigned to any linkage group.

8

9 In order to infer the relationships among the populations and the position of the root without an

10 outgroup, we first inferred a calibration-free tree using BEAST 2.1.3[38]. We used a reversible-jump

11 substitution model (RBS)[39] for each partition, which allows sampling a mixture of models, in

12 combination with a gamma distribution of rates to account for rate heterogeneity among sites.

13 We used the clockstaR 1.0 R package[40] to select the optimal number of relaxed molecular clocks.

14 This computationally efficient method computes the K-tree distance metric[41] between all

15 partition trees and uses a clustering algorithm along with a goodness-of-clustering measure (the

16 Gap statistic) to estimate the optimal number of clusters in the data[42]. We ran the analysis using

17 maximum-likelihood trees of the partitions inferred with RAxML 8.0.20[10] and used 1000

18 bootstrap replicates to estimate the Gap statistic. We found the optimal model was a single

19 molecular clock model shared by all partitions. We used a $\Gamma(\alpha = 0.001, \beta = 1000.0)$ prior

20 distribution for the clock mean rate (ucld.mean) and an $\text{Exp}(\lambda = 0.3333)$ prior distribution for the

21 clock rate standard deviation (ucld.stdev). We used a birth-death tree prior with a unif(a = 0, b =

1   1000) prior distribution of birth rates and a unif(a = 0, b = 1) prior distribution of relative death

2   rates. We applied $\Gamma(\alpha = 0.2, \beta = 5.0)$ prior distributions for RBS rates and $Exp(\lambda = 1.0)$ prior

3   distributions for the shape (α) parameter of the gamma distributions of substitution rates. We ran

4   four chains for 200,000,000 generations sampling every 5,000 generations, using the BEAGLE

5   library[43] to speed up analyses by using NVIDIA Tesla M2070 and K40 GPGPU cards for

6   parallel computation. We assessed stationarity and convergence comparing visually the

7   parameter traces with Tracer 1.6. We discarded one of the runs because it converged to a lower

8   likelihood local optimum. We removed the first 50% of samples as burn-in of the other three

9   runs and combined them with LogCombiner. Effective sample size (ESS) was over 200 for all

10   the parameters and above 4000 for the posterior and likelihood. We obtained the maximum

11   credibility tree with TreeAnnotator and summarized divergence times using the common

12   ancestor (CA) tree approach[44].

13

14   We recovered with great support (Bayesian posterior probability, BPP > 0.9) the three clades

15   reported in previous studies[45,46]: Southern Clade (comprising *T. bartmani*, *T. boharti*, *T.

16   chumash*, and *T. podura*), Santa Barbara Clade (comprising *T. cristinae*) and Northern Clade

17   (comprising *T. californicum*, *T. knulli*, *T. landelsensis*, *T. petita*, *T. poppensis*, and a putative new

18   species named *T. 'cuesta ridge'*). However, differently from some of the previous studies, we

19   inferred the root so that *T. chumash* was placed in the Southern Clade. Previous studies failed to

20   clearly resolve species relationships, especially among the species within the Northern and

21   Southern Clades, likely because the data were limited to one of a few genes[45-49]. In contrast, all

22   the species in our tree showed strongly supported reciprocal monophyly (BPP = 1), except for

23   the relationship between *T. knulli* and *T. poppensis*, where *T. poppensis* appears nested within *T.

24   knulli* as a consequence of the poorly supported (BPP = 0.63) basal placement of the *T. knulli*

25   BCE locality.

26

27   *Strategy for estimating divergence times for secondary calibration.* The fossil record of stick

28   insect is poor, and very few fossils can be unequivocally classified into any specific extant

29   lineage, and none into *Timema* in particular[50-52]. Consequently, we devised a strategy for

30   calibrating the tree of *Timema* using secondary calibrations derived from a time-calibrated tree of

31   insects. Use of secondary calibrations is preferable to extrapolating evolutionary rates, because

1   molecular rates of evolution vary across the genome[53], among lineages[54], and through time[55].

2   Moreover, similar approaches have been successfully used for mammals beforehand[56]. Our

3   strategy consisted of: (1) assembling multiple alignments of several molecular markers with

4   sequences of the main order of insects and the main clades of *Timema* retrieved from public

5   databases, (2) gathering a set of calibrations based on insect fossil data from the literature, (3)

6   inferring a time-calibrated tree of insects including divergence events for the main clades of

7   *Timema*, and (4) using divergence time estimates for such events as secondary calibrations for

8   the inference of the tree of *Timema* using the SNVs obtained from GBS data.

9

10   *Sequence retrieval and multiple alignment.* We selected the genera to be included in the

11   phylogenetic analysis of insects on the basis of: (1) their belonging to phylogenetically well-

12   supported groups (~ orders) according to the most recent review of evidence[57], (2) the

13   availability of DNA sequence data in GenBank[58], and (3) their adequacy to place calibrations

14   based on availability of reliable fossil data[50-52,59]. Likewise, we chose a range of molecular

15   markers previously used for phylogenetic inference of deep relationships among insect orders,

16   among stick insects, and among *Timema* species. In particular, we used two ribosomal

17   mitochondrial genes (12S, 16S), two protein-coding mitochondrial genes (COI, COII), two

18   ribosomal nuclear genes (18S, 28S), and three protein-coding nuclear genes (*actin*, *h3*, and

19   *hsp70*). We downloaded DNA sequences for 41 genera of insects of 13 orders. In some cases, we

20   used sequences from different species of the same genus for different markers. For every genus,

21   we chose the longest sequence when multiple accessions were available. In the case of *Timema*,

22   we used the sequences from different species to generate consensus sequences of the Northern

23   and Southern clades recovered with strong support in the previous calibration-free Bayesian

24   inference. As previously, we generated consensus sequences using a custom Perl script that

25   encoded variable positions as IUPAC ambiguities. We carried out multiple alignment of coding

26   genes using MACSE 1.01b[60], which aligns DNA sequences considering their amino acid

27   translation, followed by the elimination of all codons with over 75% gaps. We aligned rDNA

28   sequences using MAFFT 7.164b[61] with the X-INS-i alignment framework, which uses the

29   SCARNA pairwise alignment algorithm to account for RNA secondary structure[62,63]. We filtered

30   rDNA alignments with GBLOCKS 0.91b[64] setting the 'Minimum Number Of Sequences For A

31   Conserved Position' to 50% of the number of sequences + 1, the 'Minimum Number Of

1   Sequences For A Flank Position' to 50% of the number of sequences + 1, the 'Maximum Number

2   Of Contiguous Nonconserved Positions' to 10, the 'Minimum Length of a Block' to 5, and

3   'Allowed Gap Positions' to all (-b1 = 0, -b2 = 0, -b3 = 10, -b4 = 5, -b5=a). The length of the

4   alignments were: 578 bp for 12S, 651 bp for 16S, 1534 bp for COI, 696 bp for COII, 1985 bp for

5   18S, 2592 bp for 28S, 1396 bp for *actin*, 329 bp for *H3*, and 1948 bp for *Hsp70* (a total of 11,866

6   bp). We have archived the alignments in the Dryad repository.

7

8   We used PARTITIONFINDER 1.1.1[65] to select the best fit partition scheme and molecular evolution

9   model. We tested the 16 input schemes along with JC, HKY and GTR substitution models, with

10   and without gamma-distributed substitution rates, and with and without a proportion of

11   invariants (details on Dryad). We used the Bayesian Information Criterion (BIC) to select the

12   partitioning strategy best fitting the data, which was the following 6-partition scheme: (1)

13   mitochondrial rDNA (12S and 16S, 1309 bp), (2) first and second positions of mitochondrial

14   coding DNA (COI and COII, 1487 bp), (3) third positions of mitochondrial coding DNA (743

15   bp), (4) nuclear rDNA (18S and 28S, 4647 bp), (5) first and second positions of nuclear coding

16   DNA (actin, H3, and Hsp70; 2454 bp), and (6) third positions of nuclear coding DNA (1226 bp).

17   Subsequently, we used the clockstaR as before to select the optimal number of relaxed molecular

18   clocks. The optimal model was a single molecular clock model shared by all partitions.

19

20   *Calibrations.* We chose six calibrations for phylogenetically well-supported groups based on

21   robust fossil data (Supplementary Table 13). We excluded implicitly uninformative calibrations.

22   In this regard, we did not consider the only known timematodean fossil[66], which we could use to

23   calibrate the stem of *Timema*, because the stem age of *Timema* is already accounted for by

24   including an older calibration for the stem of Euphasmatodea. We defined age intervals for the

25   calibration using unequivocal fossil data to set hard lower bounds, and fossils from external or

26   more inclusive (and necessarily older) groups to set conservative soft upper bounds. For each

27   calibration, we modelled uncertainty as a gamma (Γ) probability distribution with an offset equal

28   to the minimum, a fixed-shape parameter that concentrates the mass of the distribution towards

29   the minimum ($\alpha = 2$), and a variable rate parameter (β) so that 95% of the area lies below the

30   maximum.

1   *Inference of divergence times.* We inferred a time-calibrated tree of insects with BEAST using the

2   partitioning scheme selected with PARTITIONFINDER before, but using, for each partition, a

3   reversible-jump substitution model (RBS)[39], which allows sampling a mixture of models, in

4   combination with a gamma distribution of rates to account for rate heterogeneity among sites. In

5   accordance with the results of clockStart, we used a single uncorrelated lognormal molecular

6   clock shared among all partitions. We used a $\Gamma(\alpha = 0.001, \beta = 1000.0)$ prior distribution for the

7   clock mean rate (ucld.mean) and an $\text{Exp}(\lambda = 0.3333)$ prior distribution for the clock rate standard

8   deviation (ucld.stdev). We used a birth-death tree prior with a $\Gamma(\alpha = 0.001, \beta = 1000.0)$ prior

9   distribution of birth rates and a $\Gamma(\alpha = 2.0, \beta = 2.0)$ prior distribution of relative death rates. We

10 applied $\Gamma(\alpha = 0.2, \beta = 5.0)$ prior distributions for RBS rates and $\text{Exp}(\lambda = 1.0)$ prior distributions

11 for the shape (α) parameter of the gamma distributions of substitution rates. To date the tree, we

12 placed calibrations on the stem of five well-supported groups of insects based on fossil evidence

13 from the literature (described above, see also Supplementary Table 15). Phylogenetic

14 relationships among the orders of insects are subject to intense research and some are still under

15 scrutiny. Therefore, we used the tree based on reviewed evidence from recent literature[57] as a

16 topological backbone for phylogenetic inference. We constrained the monophyly of every clade

17 supported by all five kinds of data: morphological, rDNA, mtDNA, nuclear protein-coding DNA,

18 and phylogenomic (the backbone tree has been deposited in the Dryad repository). In addition,

19 we constrained the topological relationships between the three clades of *Timema* that were

20 strongly supported in Bayesian inferences using GBS data (see above). We evaluated the joint

21 prior calibration distributions (i.e., effective priors) to ensure there were not unexpected

22 interactions among the calibrations, the birth-death tree prior, and the monophyly constraints[67].

23 We ran four chains for 10,000,000 generations adding the tag 'sampleFromPrior="true"' and

24 sampling parameters every 5,000 steps. We combined the log files with LogCombiner (part of

25 the BEAST package), after removing the first 50% of samples as burn-in, and confirmed that the

26 95% confidence intervals (CI) obtained were very similar to those of the initial Γ prior

27 distributions (Supplementary Table 13). Subsequently, we ran four chains for 100,000,000

28 generations, sampling parameters and trees every 5,000 generations, as before. We assessed

29 stationarity and convergence comparing visually the parameter traces with Tracer. We combined

30 the four runs with LogCombiner after removing the first 50% of samples as burn-in. Effective

31 sample size (ESS) was above 300 for the posterior distributions of trees and all divergence times.

1 In particular, ESS was above 800 for the distributions of divergence times we used for secondary

2 calibrations subsequently.

3

4 We estimate the median divergence time for the most recent common ancestor (MRCA) of

5 *Timema* to be 30.0 Ma (95% High Posterior Density (HPD) interval: 15.3-49.8). We estimate the

6 split between the Northern clade and the Santa Barbara clade (i.e., *T. cristinae*) to have happened

7 24.4 Ma ago (95% HPD: 10.6-42.0). This pushes the origin of *Timema* (crown-group sense) back

8 by 10 Ma when compared to the previous study, which was based on cytochrome oxidase I

9 (COI) data and the extrapolation of a generic mtDNA molecular clock rate for arthropods[47].

10

11 *Bayesian inference and divergence time estimation of localities of Timema.* We used BEAST as

12 before to carry out Bayesian inference and divergence time estimation of the populations of each

13 locality. We used the same models and priors, with the exception of the tree prior, which was set

14 to a calibrated Yule with a $\Gamma(\alpha = 0.001, \beta = 1000.0)$ prior distribution of birth rates to ensure the

15 marginal distributions of the calibrated nodes reflect the calibration priors densities[68]. We fitted a

16 Γ distribution to the posterior distributions of divergence times estimated previously with the

17 function "fitdistr" of the MASS R package 7.3-29[69] and used them as calibration priors.

18 Specifically, we placed a $\Gamma(\alpha = 12.757, \beta = 2.326)$ distribution on the split between the Southern

19 clade and the Northern and Santa Barbara clade (effectively the root), and a $\Gamma(\alpha = 9.791, \beta =$

20 2.432) distribution on the split between the Southern clade and the Santa Barbara clade (see

21 Supplementary Table 14 for details). We ascertained the effective prior distribution of the

22 calibrations as before, but because of problems related to chains being invariably trapped in

23 infinite log-likelihood values after a few million generations, we ran instead 40 chains for 1

24 million of generations, sampling every 5000 and combined them using LogCombiner after

25 removing the first 10% as burn-in. We confirmed that there were no unexpected interactions

26 among the priors (Supplementary Table 14). We then ran 14 chains for 200,000,000 generations,

27 sampling every 5,000 generations. After careful visual examination of the traces with Tracer, we

28 retained four runs that consistently converged onto the same stationary distribution for multiple

29 parameters and showed the highest mean posterior probability and likelihood. We removed the

30 first 75% of samples as burn-in and combined them with LogCombiner. ESS was over 200 for

31 most of the parameters of the posterior distribution, and, in particular, it was above 3000 for the

1 tree likelihood distributions. We obtained the maximum credibility tree with TreeAnnotator and

2 summarized divergence times using the common ancestor (CA) tree approach[44].

3

4 **Supplementary Table 13.** Fossil evidence used for calibration. Uncertainty was modelled as a

5 gamma (Γ) distribution with an offset (see text for details). The effective prior distributions are

6 summarized given the 95% confidence interval. Ma = millions of years.

Stem group	Justification		Age range (Ma)	BEAST calibration	Effective prior (Ma)	References
	Minimum	Maximum				
Entognatha	Oldest hexapodan fossil: *Rhyniella praecursor* (Entognatha) from the Lochkovian-Pragian (Lower Devonian)	Cambrian explosion	419-541	Γ(α=2, β=25.7); o=419	420.6-530.0	[51,70,71]
Holometabola	Oldest insect gall fossil trace from the Upper Pennsylvanian (Upper Carboniferous)	Split between Entognatha and Insecta	302-419	Γ(α=2, β=24.6); o=302	304.9-398.3	[72]
Diptera	Oldest dipteran fossil: *Grauvogelia arzvilleriana* from the Lower Anisian (Middle Triassic)	Split between Holometabola and rest of Insecta	247-302	Γ(α=2, β=11.5); o=247	247.1-283.5	[73]
Holophasmatodea	Oldest stem-phasmatodean fossil: *Cretophasmomima melanogramma* from the Yixian formation (Lower	Split between Entognatha and Insecta	129-419	Γ(α=2, β=40.0); o=129	131.5-272.5	[59]

Cretaceous)					
Euphasmatodea	Oldest euphasmatodean fossil eggs from the Cenomanian (Upper Cretaceous)	Oldest stem-phasmatodean fossil: *Cretophasmomima melanogramma* from the Yixian formation (Lower Cretaceous)	95-129	Γ(α=2, β=7.1); o=95	96.1-150.5 [50,51,59,74,75]
Phylliidae	Oldest leaf insect fossil: *Eophyllium meselensis* from the lower Middle Eocene	Origin of the MRCA of Euphasmatodea	47-95	Γ(α=2, β=10.1); o=47	47.7-82.3 [52]

1

2 **Supplementary Table 14.** Secondary calibrations used to date the tree of *Timema* populations.

3 Ma = millions of years.

Split	BEAST primary estimation (Ma)	BEAST secondary calibration	BEAST secondary calibration range (Ma)	Effective prior (Ma)	BEAST secondary estimation (Ma)
Timema root	15.3-49.8	Γ(α=12.2, β=2.6)	17.0-53.1	19.6-51.9	19.1-47.6
Northern Clade – Santa Barbara Clade	10.6-42.0	Γ(α=9.6, β=2.7)	12.6-45.8	10.9-35.4	13.2-35.0

4

5 **Supplementary Figure 4. Maps of the study localities used in the different analysis.** (A) *T.*

6 *cristinae*. (B) Genus-wide. (C) Cline. (D) Map of species ranges. Modified from [45].

7

A Populations of *Timema cristinae* used for genome-wide differentiation analysis

B Populations of *Timema spp.* used for genome-wide differentiation analysis

C Populations of *Timema cristinae* used for allelic cline analysis

D Species distribution map
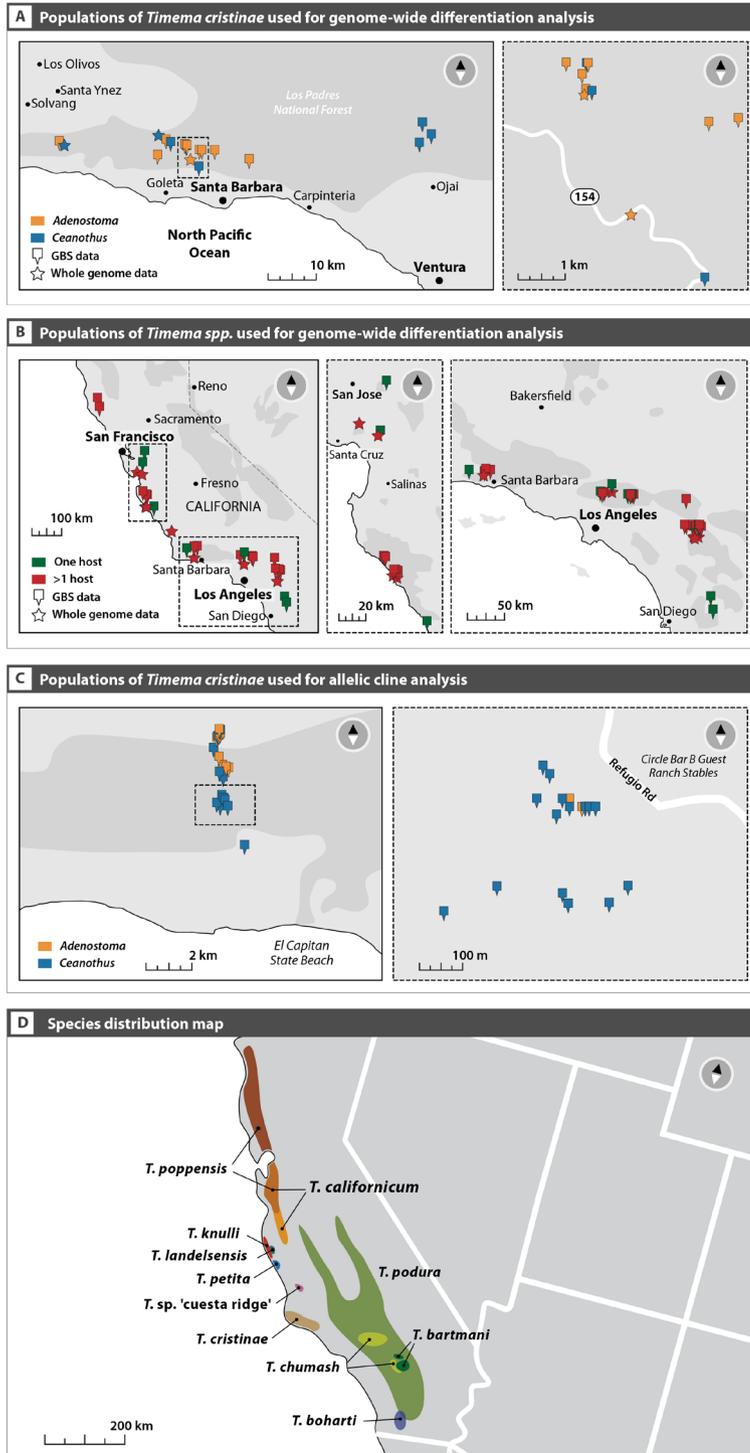
1

2

1 **Supplementary Table 15.** Summary of data that were re-analysed from previously published

2 studies and that are new to this study. GWA = Genome wide association. HMM = Hidden

3 Markov Model.

Analysis	Phenotypic data	Genomic data
Quantification of cline in morph frequencies	New to this study	N/A
GWA mapping of colour-pattern	From[21]	From[21]
Whole genome HMM analysis of accentuated and background differentiation in *T. cristinae* (160 genomes)	N/A	From[1]
Whole genome HMM analysis of transplant-and-sequence experiment (473 genomes)	From[37]	New to this study
Association of population differentiation in colour-pattern with genomic differentiation	New to this study	New to this study
GWA mapping of CHCs	From[21]	From[21]
Perfuming experiment	New to this study	N/A
Association of population differentiation in CHCs with sexual isolation	CHCs new to this study; sexual isolation data from[76]	N/A
Association of population differentiation in CHCs with genomic differentiation	New to this study	New to this study
Whole genome HMM analysis if accentuated and background differentiation in multiple *Timema* ecotypes and species (379 genomes)	N/A	New to this study
Genome-wide differentiation between sympatric ecotypes and species using GBS data	N/A	New to this study
Temporal evolution of sexual isolation between species	From[22]	New to this study
Temporal evolution of morphological differentiation between species	New to this study	New to this study

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