Ecography

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Supplementary material

Appendix 1. Instructions for making measurements on iNaturalist photos using ImageJ

• Download and install ImageJ from https://imagej.nih.gov/ij/download.html
• Opening images: Drag JPEG file onto the ImageJ icon on the sidebar.
• Optimizing view: You can re-size the image at any time to make better measurements; from the keyboard, use + to enlarge or - to shrink. To reposition the image, use the space bar to select the hand tool.
• Preparing macro: Copy and paste the macro script provided in Appendix S2 into a blank text file. Save macro file as 'photograph_macro.txt'.
• Loading measurement tools: Go to Plugins > Macros > Install in the pulldown menu and open 'photograph_macro.txt' The macros will then be listed under Plugins > Macros, but you can run them by pressing the numbers shown.

First, measure the entire available wing surface:
• Setting the (arbitrary) scale: Before you can make area measurements, you need to set the scale of the image. For perching photos, there is no scale bar to use, so as a workaround, just use the line tool to draw a line across the individual's eye. Then, press 1 (here and below, the number must be entered on the upper keyboard strip and not in the number keypad to the right) and then 1 again to set the scale of the image.
• Use the polygon tool to make area measurements 10 (the entire outline of all wings visible) and 11 (the entire outline of the wing spot [the basal wingspot in titia]) and 12 (the tip spot in titia). After you outline the area, press 1 and then enter the measurement code (see diagram).
• To change the code of a measurement, press 2 and then enter the number of the measurement and the new code.
• If you make a bad measurement, use the re-coding tool [2] to change the code of the bad measurement to 9 to tag it for deletion in excel. Then remeasure the area.
• Once you have taken all of the measurements, press 9. This copies the results to the clipboard for transfer to a datasheet and saves the analysed image file with a "z" before the name. IMPORTANT: To this file name, add a 'z' to the end of the image file, so that this photo is saved as 'zz'.

Second, measure the hindwing surface only:
• Re-open the original image file, and repeat the procedure on the HINDWING only (1, then 10 [the outline of the entire hindwing surface], then 11 [the wingspot], and 12 [if present]), then save. When you finish and save this photo, there will be two new images corresponding to this file: 'z' (hw measured) and 'zz (whole wing surface measured)' files for each photo.

Appendix 2. Macro script written for measuring photographs in ImageJ

```
//Global variables
var n = 1;
var scale = 0;
var length = 0;

macro "TAKE MEASUREMENT [1]" {
       type = selectionType();
       if (type==-1)
      exit("You need to select something first.");
      code = getNumber("code:", code);
       if (code>23)
       exit("Invalid code. This version of the macro recognizes codes 1-23.");
       if (code<1)
       exit("Invalid code. This version of the macro recognizes codes 1-23.");
       if (code==1) {//set scale
       //so that Measure yields area and xy:
       if (type!=5)
             exit("Error: use line tool to set scale");
       run("Set Measurements...", "area centroid redirect=None decimal=5");
       run("Clear Results");
       n =0;
       getLine(x1, y1, x2, y2, lineWidth);
       if (x1==-1)
      exit("Error: use line tool to set scale");
      scale = 2; // for a 2 mm scale line
       unit = "mm";
       dx = x2-x1;
       dy = y2-y1;
       length = sqrt(dx*dx+dy*dy);
       run("Set Scale...", "distance="+length+" known=scale pixel=0.99613 unit="+unit);
       // REM to set pixel to pixel aspect ratio for monitor
}
       if (scale==0)
       exit("Need to set scale first, using 2 mm on scale bar.");
       if (code!=1 && type!=2)
              exit("Use polygon tool to measure areas.");
       run("Measure");
       setResult("Scale", n, length);
       setResult("Code", n, code);
       setResult("Image", n, getTitle);
       n = n+1;
       run("Overlay Options...", "stroke=yellow width=5 fill=none set");
       run("Labels...", "color=white font=14 show bold");
       run("Add Selection...");
}

macro "RECODE [2]" {
rn = getNumber("Measurement you want to recode (esc to cancel):", n);
if (rn==1)
       exit("You can't recode the scale line.");
code = getNumber("New code:", code);
if (code>23)
       exit("Invalid code. This version of the macro recognizes codes 1-23.");
if (code<1)
       exit("Invalid code. This version of the macro recognizes codes 1-23.");
if (code==1)
       exit("You can't recode a measurement with the scale line code.");
setResult("Code", rn-1, code);  //rn-1 because first measurement is n = 0
}

macro "COPY RESULTS AND SAVE IMAGE [9]" {
String.copyResults
getDateAndTime(year, month, dayOfWeek, dayOfMonth, hour, minute, second, msec);
mo = month+1 // because Jan = 0
timestring = "Measurement date: " + year +" "+ mo +" "+ dayOfMonth;
setFont("SansSerif", 112, " antialiased");
setColor("black");
drawString(timestring, 102, 166);
//makeText(timestring, 102, 166);
path =  getDirectory("image");
zfile =  "z" + getTitle;
saveAs("Jpeg",  path + zfile);
}
```

Table A1. Study locations where standard and perching photos were taken for proof-of-principle analyses.

species	site code	latitude	longitude
H. titia	LCPC1	31.2792	-89.7131
	BF17	31.0749	-93.4959
	NPKB1	31.5166	-93.1433
	LCSB3	31.3222	-89.7011
	LCBC4	31.2756	-89.7311
	WCKC1	31.2692	-90.1692
	PCCC1	30.988	-89.0057
	TPAR1	30.7231	-90.1408
C. splendens	YODE01	53.84	-0.946
	LIID01	53.457	-0.8484
	NOID01	53.3629	-0.9289
	NHWE01	52.519	-0.7331
	NHIS01	52.4123	-0.693
	SHRT01	52.3094	-2.6662
	CATS01	52.3258	-0.1097
	NHRT01	52.1324	-0.9603
	CDRW01	54.7657	-1.5542

Table A2. Linear regressions, modelling indices of relative wing pigmentation as measured from standard photographs (Y) as a function of indices measured on perched photographs (X).

species	measurement	model term	estimate	SE	t-value	p-value
H. titia	hindwing	intercept	-0.05	0.02	-3.12	0.003
		X	1.02	0.02	48.53	< 0.001
					Adjusted R^2:	0.97
	entire wing	intercept	-0.06	0.02	-3.92	< 0.001
		X	0.74	0.02	34.34	< 0.001
					Adjusted R^2:	0.95
C. splendens	hindwing	intercept	-0.14	0.02	-6.12	< 0.001
		X	1.14	0.04	28.15	< 0.001
					Adjusted R^2:	0.89
	entire wing	intercept	-0.13	0.02	-5.75	< 0.001
		X	1.22	0.04	29.12	< 0.001
					Adjusted R^2:	0.90

Table A3. Spearman rank correlations measuring inter-observer reliability in different measurement types.

species	**photo**	**hindwing *ρ* (n)**	**entire wing *ρ* (n)**
H. titia	standard	0.98 (32)	0.99 (32)
	perching	0.98 (27)	0.97 (28)
C. splendens	standard	0.99 (29)	0.98 (29)
	perching	0.88 (36)	0.94 (35)

Table A4. Mixed-effect regression of the proportion of the visible wing surface with pigment in *H. titia* males. The dependent variable was log-transformed, and longitude, latitude, julian date and julian date were standardized to a mean of zero and variance of 1, prior to analysis. Samples with the same longitude and latitude (rounded to two decimal places) were grouped together under a random effects "site" term, to eliminate spatial autocorrelation (n = 426 samples, 266 groups), the absence of which was verified with Moran's I (observed = -0.0149, expected = -0.0027, sd = 0.0309, $p = 0.69$). The 95% confidence intervals (calculated from the likelihood profile, using the confint function in lme4) for terms presented in bold do not cross 0 (i.e., in this model, no terms' confidence intervals crossed 0). A model excluding regional groupings provided a far worse fit to the data than the model presented here ($AIC_{[\text{model with 'region'}]} - AIC_{[\text{model without 'region'}]} = -91.22$). Tukey's method was used for the multiple comparisons between regions.

a. fixed effects

model term	**estimate**	**confidence int.**	
		lower	**upper**
intercept	**-0.57**	**-0.63**	**-0.52**
Northern region	**-0.96**	**-1.17**	**-0.75**
Pacific region	**-0.48**	**-0.73**	**-0.24**
Julian date	**1.34**	**1.16**	**1.54**
(Julian date)^2	**-1.49**	**-1.68**	**-1.30**
latitude	**-0.09**	**-0.15**	**-0.03**
longitude	**-0.08**	**-0.15**	**-0.01**

b. multiple comparisons

contrast	**estimate**	**std. error**	***z*-value**	***p*-value**
Northern-Atlantic	-0.96	0.11	-8.73	<0.001
Pacific–Atlantic	-0.48	0.13	-3.81	<0.001
Pacific-Northern	0.48	0.19	2.49	0.029

Table A5. Mixed-effect regression of the log-transformed proportion of the hindwing surface with pigment for *H. titia* males (n = 437), (a) excluding the 'region' variable from the main text and (b) including the Pacific/Atlantic regions, but lumping "northern" observations into the "Atlantic" region. Julian date and its quadratic term, latitude, and longitude were all z-transformed prior to model fitting. A 'site' variable was included as a random effect (see Table 2 caption). The 95% confidence intervals (calculated from the likelihood profile, using the confint function in lme4) for terms presented in bold do not cross 0 (i.e., in these models, no terms' confidence intervals crossed 0). ΔAIC values presented are calculated as $AIC_{[model\ with\ 'region']} - AIC_{[model]}$, where the model with 'region' refers to the model presented in Table 2.

model	model term	estimate	confidence int. lower	confidence int. upper
(a) no region	**intercept**	**-0.85**	**-0.91**	**-0.79**
ΔAIC = -91.63	**Julian date**	**1.79**	**1.56**	**2.02**
	(Julian date)^2	**-1.94**	**-2.17**	**-1.72**
	latitude	**-0.21**	**-0.27**	**-0.15**
	longitude	**-0.36**	**-0.42**	**-0.29**
(b) Pacific/Atlantic regions	**intercept**	**-0.81**	**-0.87**	**-0.76**
	Pacific region	**-0.86**	**-1.16**	**-0.55**
ΔAIC = -68.09	**Julian date**	**1.56**	**1.32**	**1.80**
	(Julian date)^2	**-1.74**	**-1.97**	**-1.51**
	latitude	**-0.28**	**-0.34**	**-0.21**
	longitude	**-0.33**	**-0.39**	**-0.26**

Table A6. Mixed-effect regression of the log-transformed proportion of the hindwing surface with pigment for *H. titia* males, binning observations for each month to test for an effect of observation rate on inferences ($\underline{n}$ = 315 site-by-month observations). Month, latitude, longitude, and the number of observations were all z-transformed prior to model fitting. A 'site' variable was included as a random effect (see Table 2 caption). The 95% confidence intervals (calculated from the likelihood profile, using the confint function in lme4) for terms presented in bold do not cross 0. A model excluding the region term provides a poorer fit, confirming an impact of region ($AIC_{[\text{model with 'region'}]} - AIC_{[\text{model without 'region'}]}$ = -110.43).

model term	**estimate**	**confidence int.**	
		lower	**upper**
intercept	**-0.55**	**-0.63**	**-0.47**
Northern region	**-1.09**	**-1.40**	**-0.78**
Pacific region	**-1.32**	**-1.65**	**-0.99**
month	**-0.18**	**-0.25**	**-0.11**
latitude	-0.08	-0.18	0.01
longitude	-0.06	-0.16	0.03
observation count	0.02	-0.04	0.08

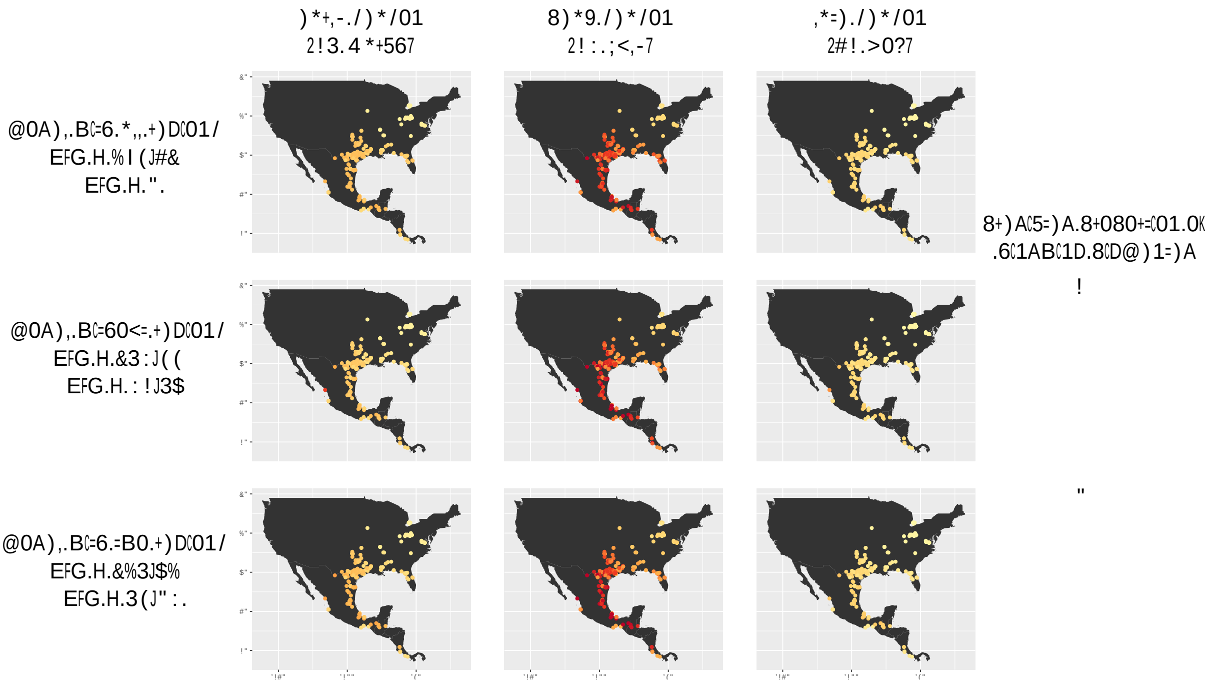

Figure A2. Predicted levels of wing pigmentation in the early season (Julian date = 75), peak season (Julian date = 200) and late season (Julian date = 325) from each of the three models with different region categories. Predictions across all models are largely concordant, but a model with discrete regions for Atlantic, Pacific, and northern populations (top) greatly outperforms models with only latitude and longitude (middle) or a model that lumps Atlantic and northern populations (bottom). Predicted values larger than 1 were set to 1 for plotting.