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A METHOD FOR THE QUANTITATIVE ANALYSIS OF WING SPOTS APPLIED TO TWO POPULATIONS OF *CALOPTERYX SPLENDENS* (HARRIS) (ZYGOPTERA: CALOPTERYGIDAE)

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The coloured wing spot on a sample of males from 2 populations of *C. splendens* s.l. (Boutonne, France; Ourthe, Belgium) are analysed, using an iterative computation procedure on wing images transferred to a computer memory by a video transducer. The analysis reveals significant differences in apical wing spot extent and total wing spot area, but not in basal wing spot extent. Its sensitivity is such that it permits to identify immigrants, thus potential gene flow, to the native population. The new possibilities for analysis of the complex of *C. splendens* are briefly discussed.

INTRODUCTION

In damselflies where reproductive isolation is ensured by a pre-mating behavioural display, selective pressure is removed from mechanical isolational mechanisms, and the classical lock-antilock system does not function. Structural characters, especially if valid in the two sexes, are much easier to work with for taxonomists than the more esoteric patterns of behaviour, and this has resulted in unsatisfactory classifications.

Calopteryx splendens s.l. is a case in point. The range of this taxon is enormous, and encompasses almost the whole of Europe, large portions of Siberia (as far as, roughly, 60°N) and much of west and central Asia (ASKEW, 1988; BELYSHEV, 1973; BARTENEFF, 1912, 1930). Across this vast territory, it displays a remarkable constancy in structural characters (male appendices, hamuli, and copulatory organ; female pronotum and adjacent synthorax, and ovipositor), but considerable variation in precopulatory display of males towards females (BUCHHOLTZ, 1951, 1955; HEYMER, 1972). It is generally accepted that the extent of a coloured spot on all four wings acts as a mate recognition signal to the female (the pigment involved is a brown ommochrome, which interacts with the metallic colours of the main veins in flying specimens, to give an impression of a purple metallic spot). The wing spot varies tremendously between subzones of the species range, but is generally more constant within than between the populations, suggesting that there is a genetic component to it, and that gene flow in these insects is not absent, but limited by environmental topography (DUMONT, 1987). A complex mosaic-shaped pattern of wing-spot types emerges, with the wing spot present at eclosion, or only appearing several days later (DUMONT, 1972). All possible extremes (no wing spot at all, or wing entirely covered by the spot) occur, some even several times, often in widely disjunct locations (DUMONT, 1987).

As the wing spot in males becomes larger, there is a tendency for it to occur in females (normally having fully hyaline wings) as well (the so-called homochrome females). Although the genetics behind the wing-spot development remain obscure, this is suggestive of negative regulation by gene-titration of the genes expressing the wing spot, with suppressor genes located on the X chromosome.

The subject of the present study is concerned with developing an exact method for measuring the phenotypic expression of the wing spot at the population level. This is a first, but necessary step, towards an objective comparison of populations, eliminating the need to describe wing spots as either "big", "small", or "average". We offer a quantitative measure instead, with means and standard deviations, making the analysis amenable to an evaluation by statistical tools.

MATERIAL AND METHODS

The present paper is aimed at demonstrating the capabilities of wing image storage and subsequent mathematical manipulation of wing shape variables in comparing a medium-sized sample from two distinct populations. The indicated statistic is thus paired t-testing, but extensions of the method to more populations (presently underway) will require ANOVA or MANOVA techniques.

The first population sampled was at the River Boutonne at Dampierre-sur-Boutonne, near St. Jean d'Angély, France (46° 08' N, 0° 13' W), 10 Sept. 1988. This is exactly the site where LACROIX (1919) collected his *C. splendens faivrei* (DUMONT, 1971). From this Boutonne population, 28 males were used for wing spot analysis.

A presumed 'typical' population of *C. splendens* was sampled at Bas Rendeux near Hotton, Belgium (50° 16' N, 5° 27' E), situated on the River Ourthe, 15 July 1988. From this Belgian population, 24 males were analysed.

As a first step, an entire wing was scanned into the memory of an automated image analysis system (Quantinet 970), using a black and white video camera. Enlargement and light conditions were first fully standardized. In a second step, a full set of coordinates (see further) of the perimeter of each individual wing were measured and stored. The software used was Plan Perfect version 5.1, supplemented by an algorithm developed by the third author. In this, an initial interface procedure ensured that per scanned line (see hereafter), there is always an even number of perimeter points, some of which may coincide, which is delivered to the different software procedures which take care of the calculation of the parameters required.

PRINCIPLES OF WING-SPOT ANALYSIS

A coordinate system is so selected that its origin coincides with the nodus (Fig. 1a), because the nodus is a constant and well-defined reference point. The abscissa runs along the postnodal costal vein, with positive values along that axis situated apicad. The ordinate crosses the wing perpendicularly.

A series of features of the wing spot can now be quantified using this axis, and expressed in function of its total surface. To make it possible to compare wings of different individuals (different sizes), standardized length values (x,y) were preferred to absolute values. The standard length on the axis was defined as the distance nodus-apex, which is set equal to 100. A wing is divided in about 300 transects t (measured along the y-axis). The length of each transect equals 100, by definition, and the share of the wing spot in it is therefore automatically expressed as a percentage. This procedure permits to express the relationship of the extent of a wing spot (y) to wing length (x) uniformly for all wings (Fig. 1b).

This iterative procedure, programmed to be run automatically, leads to the following parameters of interest:

$x_{50,min}$,$x_{50,max}$: the x-values for which $y = 50$

x_{min}: the transect where a wing spot begins, or the lowest value of x for $y > 0$.

x_{max}: the transect where a wing spot ends, or the highest value of x for $y > 0$.

L_m: the modal length of the spot; $L_m = x_{50,max} - x_{50,min}$

L_x: the maximum length of the spot; $L_x = x_{max} - x_{min}$

B/L: the relative width of the wing; the relation width of the wing, or the longest transect, divided by the standard length of the wing.

The change Δy of the function y, for a given increment Δx is expressed as $y' = \Delta y/\Delta x$. It is calculated for any $\Delta y = y_n - y_{n-1}$, with $\Delta x = x_n - x_{n-1}$, and for each of n transects ($0 < n < 300$). Figure 1c shows the behaviour of y' for two sets of nonzero y'-values (v_1 and v_2). The course of y' gives an impression of the slope and the regularity/irregularity of the wing spot.

The following values of interest can be read from this graphical presentation:

y'_1 and y'_2: the highest value of y^1 within v_1 and v_2. They indicate the site where the border of the spot has the highest slope.

$v_{1,m}$ and $v_{2,m}$: the x-values corresponding to y'_1 and y'_2.

$v_{1,s}$ and $v_{2,s}$: the dispersion of v_1 and v_2 expressed as:

$$\left| v_{1,max} \right| - \left| v_{1,min} \right| ; \left| v_{2,max} \right| - \left| v_{2,min} \right| ,$$

with $v_{1,max}$, $v_{1,min}$, and $v_{2,max}$, $v_{2,min}$ the extreme (limit) values of v_1 and v_2 on the x-axis. These values permit the identification of different wing spot types. A short and smoothly rounded spot results in a high y_1 value, a low $v_{1,s}$ and $v_{1,m}$ centrated.

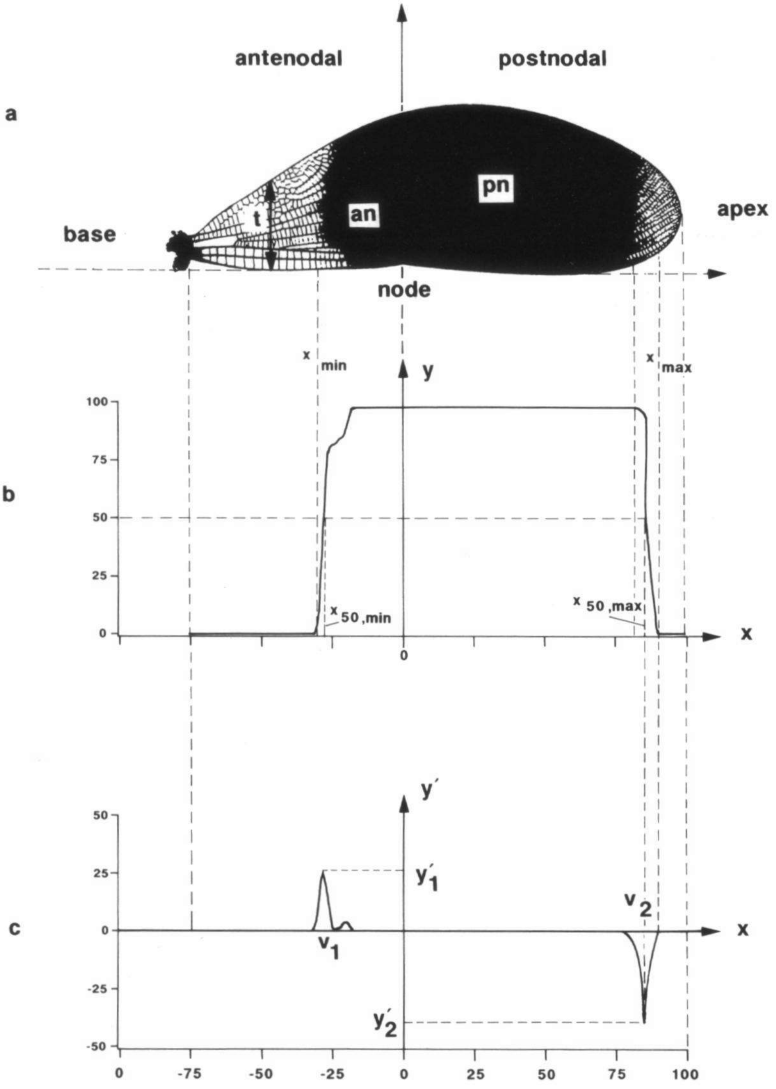

Fig. 1. Principle of wing-spot analysis: (a) orientation of the wing in a coordinate system; – (b) the relationship of the extent of the wing spot y to wing length x; – (c) the change y' of the previous function for an increment of x, which gives an impression of the regularity of the wing spot boundaries.

Integration of the function y amounts to calculating the wing spot area, in relation to wing area. Conventionally, we equate the postnodal wing surface area P to 100. The integration leads to the following relevant parameters (Fig. 1a):

an: antenodal spot surface area.
pn: postnodal spot surface area.
t: total spot area (t = a+p).
t/T.100: the relation of the spot surface to total wing surface area (in percent).

A COMPARISON OF THE TWO POPULATIONS

The wing spot pattern is characterized by two features: the localisation of the proximal and apical borderlines of the ommochrome pigment. We here test our method of wing spot analysis in a comparison of males of two populations: *C. splendens faivrei* from the river Boutonne, and *C. s. splendens* from the river Ourthe (Tabs I, II, III).

Tables I and II show that all values which relate to the proximal border of the spot ($x_{50,min}$; x_{min}; $v_{1,s}$; y^{1}_{1}; $v_{1,m}$) are not significant (t-test), i.e. neither shape nor position of this line are different between the two populations considered (Fig. 2).

This is illustrated further in Figure 3, which displays wings with extremes (min, max) for x_{min} from Table I. Variation in the proximal borderline is such that the minimum values (Fig. 3 a,b) and maximum values (Fig. 3 c,d) are closer between than within populations.

In contrast, the position of the apical borderline between the two populations is highly significantly different ($p < 0.001$). It is displaced apicad in the Boutonne population ($x_{50,max}$: 85.21 length units per 100) relative to the Ourthe population ($x_{50,max}$: 76.29). The same result is true for x_{max} and $v_{2,s}$. It follows (Tab. III) that the postnodal wingspot area is larger in the Boutonne population (pn = 88.95)

Table I

x, y values

n	Code	$x_{50,min}$	$x_{50,max}$	x_{min}	x_{max}	y_{max}	L_m	L_x	B/L
24	mean	-14.33	76.29	-18.25	77.96	97.89	90.63	96.21	0.67
	s.d.	5.20	3.47	4.53	3.80	1.03	7.03	6.68	0.04
	min	-23.00	70.00	-27.00	71.00	95.27	72.00	79.00	0.58
	max	- 2.00	81.00	- 8.00	83.00	100.66	103.00	109.00	0.74
	var.	36.26	4.55	24.84	4.88	1.05	7.76	6.94	6.17
28	mean	-16.61	85.21	-20.61	86.36	98.72	101.82	106.96	0.63
	s.d.	4.46	3.71	5.24	3.82	0.60	5.78	6.39	0.04
	min	-25.00	73.00	-37.00	75.00	97.89	92.00	96.00	0.55
	max	-10.00	92.00	-12.00	93.00	100.26	112.00	123.00	0.76
	var.	26.85	4.35	25.42	4.43	0.60	5.68	5.97	6.02
	t	<0.1	5.475	<0.1	4.452	<0.1	2.730	2.427	0.120
	p	n.s.	0.001	n.s.	0.001	n.s.	0.02	0.05	n.s.

Table II

Derivatives (x', y')

n	Code	$v_{1,s}$	$v_{2,s}$	x^1	$v_{1,m}$	y^1	$v_{2,m}$
24	mean	5.25	5.21	15.22	-16.25	-25.73	76.83
	s.d.	1.67	1.18	3.62	5.80	5.35	3.36
	min	3.00	3.00	9.48	-26.00	-38.78	70.00
	max	10.00	8.00	22.06	- 1.00	-16.16	82.00
	var.	31.90	22.63	23.78	35.71	20.78	4.37
28	mean	5.93	4.46	17.07	-18.57	-30.42	85.61
	s.d.	1.49	0.84	4.91	4.06	5.91	3.70
	min	3.00	3.00	10.40	-26.00	-39.80	74.00
	max	9.00	7.00	27.66	-12.00	-16.88	92.00
	var.	25.11	18.77	28.79	21.86	19.44	4.32
	t	<0.1	<0.1	<0.1	<0.1	<0.1	5.489
	p	n.s.	n.s.	n.s.	n.s.	n.s.	0.001

Table III

Surface areas

n	Code	T	t	A	an	pn	t/T.100
24	mean	149.62	94.16	49.62	12.87	81.29	62.90
	s.d.	3.86	7.21	3.86	4.72	4.45	4.12
	min	142.76	76.84	42.76	3.38	73.29	53.82
	max	158.31	107.26	58.31	21.70	94.85	72.23
	var.	2.58	7.66	7.77	36.66	5.47	6.55
28	mean	151.94	104.61	51.94	15.64	88.95	68.87
	s.d.	3.36	4.92	3.36	4.10	2.87	3.29
	min	146.47	95.93	46.47	9.77	79.06	62.71
	max	158.10	113.55	58.10	24.41	93.52	76.49
	var.	2.21	4.70	6.47	26.18	3.23	4.78
	t	<0.1	2.528	<0.1	<0.1	3.849	3.437
	p	n.s.	0.02	n.s.	n.s.	0.001	0.001

than in the Belgian population (pn = 81.29), but not the antenodal part of the spot (a), while total wingspot area (t) is again significantly larger in the Boutonne (68.87%) than in the Ourthe population (62.90%).

DISCUSSION

The analysis presented here leads to several non-trivial conclusions. For example, we clearly show that *C. s. faivrei* differs from *C. s. splendens*, but this

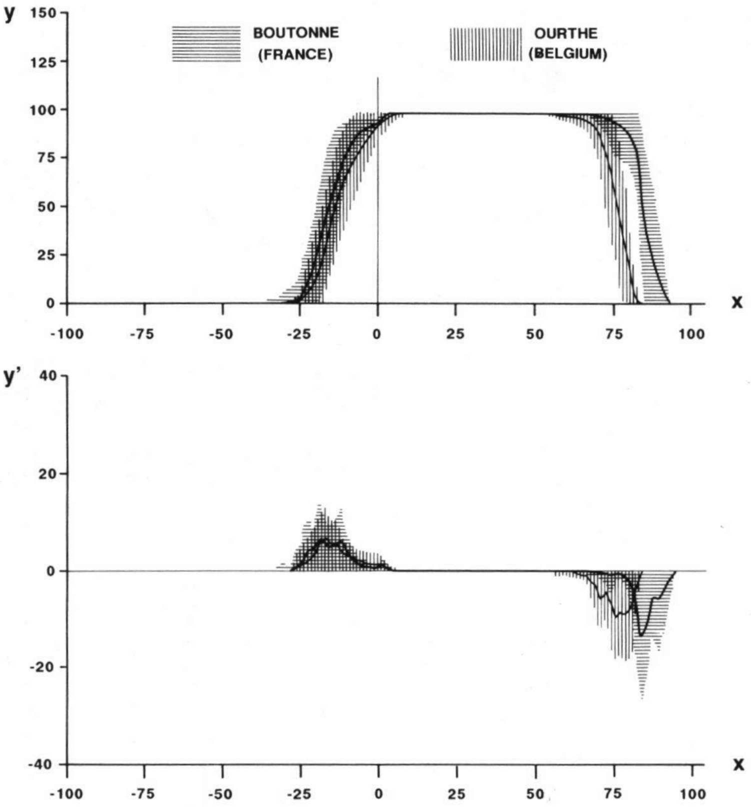

Fig. 2. Wing-spot analysis of two populations as explained in the text.

difference is solely due to the position of the apical borderline of the wingspot, which, in its turn, determines total wing spot length and surface area. The basal wing spot delimitation and the wing width (B/L) do not permit to distinguish both populations.

The analysis also shows that the standard deviation of $x_{50,max}$ is a measure of the variability of the position of the borderline. By definition, 68% of all values are comprised within $x_{50,max}$ ± s.d., assuming a normally distributed population. This implies that in the Belgian population, variation of the position of the distal borderline is 3.47 units (3.47% of the distance nodus-apex) (Tab. I). The variation of the same feature of the Boutonne population is 3.71%, and is not statistically significant, unlike suggested by DUMONT (1972). This illustrates the advantage of a quantitative over a qualitative approach.

Furthermore, and again by definition, 95% (p = 0.05) of the values for the apical borderline fall within $\bar{x}_{50,max}$ ± 1.96 s.d., or 85.21 ± 7.27 (Fig. 3). An inspection of Figure 4 shows that one Boutonne specimen with $\bar{x}_{50,\max}$ = 73.00 is below the limit value of 77.94. This suggests that we might have an immigrant from a typical *C. splendens* population here.

Quantitative wingspot analysis thus opens several new possibilities: to quantify and compare wingspot sizes, to analyse genetical variation of this trait, and to measure allochthonous gene inflow. We hypothesize that, by extending the analysis to the entire range of the *C. splendens*-complex, we should be able to arrive at an objective classification of the various groups of populations in subspecific and specific taxa. If a decision about where to draw the line between taxa will still be arbitrary, it will at least be quantitative.

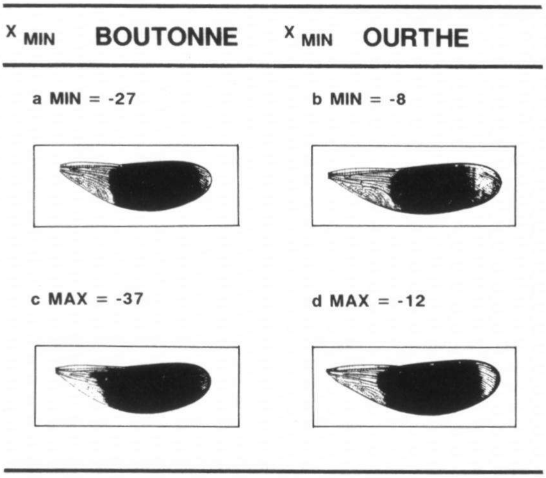

Fig. 3. Population extremes (min, max- of wing-spots in both populations (Boutonne, Ourthe); explanation see text.

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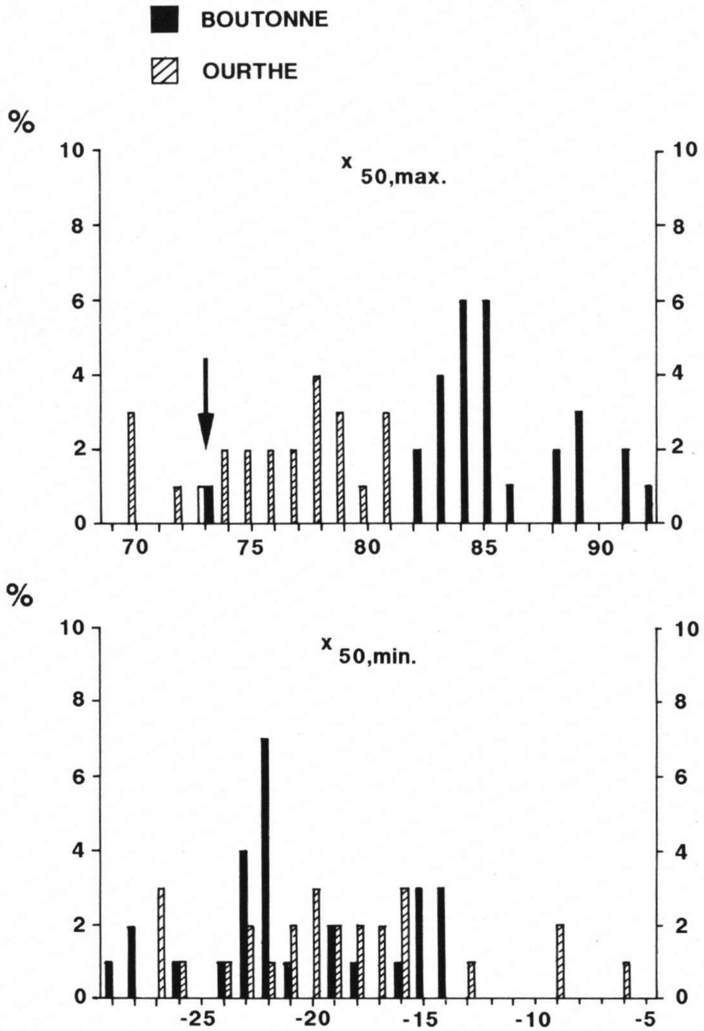

Fig. 4. Frequency distribution (%) of $\bar{x}_{50,max}$ and $\bar{x}_{50,min}$ values for both populations. – [Arrow on upper graph indicates an immigrant to the Boutonne population].