

Data concerning the relative distribution of two varieties of *Discoglossus pictus* in Sicily (*Amphibia*)

by

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This paper is dedicated to Prof. F. Baltzer as an acknowledgement of his great contribution to zoological research.

INTRODUCTION

Discoglossus pictus is an anuran which is very common in Sicily; its eggs can be obtained at practically any time of the year, so it is an excellent material for embryological investigation.

The genus is distributed in a rather limited area: the south eastern region of France (eastern Pyrénées), Spain, north Africa (Maroque, Algerie, Tunisia), Sicily, Sardinia and Corsica with some closely related islands.

Until a few years ago only one species, *pictus* (with two subspecies) was included in this genus: however recently MENDELSSOHN and STEINITZ (1943), added a new species, *nigriventris*, from Palestine; and more recently KNOEPFFLER (1961) maintained that the subspecies *sardus* from Sardinia and Corsica is to be elevated to a species. According to KNOEPFFLER, then, the gen. *Discoglossus* would include 3 species: *pictus* distributed on a rather large area;

sardus limited to Sardinia, Corsica and some of their contiguous islands; and finally *nigriventris*, limited to a very narrow locality in Palestine. The morphological and ecological characteristics of these 3 species have been accurately described by various authors.

The species *pictus* was described by OTTH in 1837, on a few animals captured in Sicily and kept in the Museum of Vienna. Authors agree that the coloration of the skin is highly variable; in this respect, CAMERANO (1883) distinguished two varieties: *D. pictus* var. *vittata*, and *D. pictus* var. *ocellata*. SCHREIBER (1912) added another variety characterized by red-brown, uniform coloration.

Data concerning the relative frequency of these varieties in a particular area, or in different countries, are very scarce: according to LANTZ (1947) "In *D. pictus*, striped and spotted specimens occur side by side throughout the range, without, as far as is known, any marked preponderance of the one form on the other". In Sardinia and Corsica the var. *vittata* is absent (LANTZ, 1947). KNOEPFFLER (1961) also notes that the specimens of those regions are always spotted.

Until recently it was supposed that the diversity of coloration was due to environment. However LANTZ (1947), GALLIEN (1948), ALONSO-BEDATE (1960) and KNOEPFFLER (1961) showed that the type of coloration is genetically determined. This conclusion is also supported by the results obtained by ALONSO-BEDATE (1959) and LA SPINA (1963), transplanting fragments of the neural crests (which are responsible for the colored pattern) from *vittata* to *ocellata*, and vice versa.

2. MATERIAL

The material under examination consisted of adults and recently metamorphosed individuals from different localities in Sicily. The sex of adult specimens was determined from observations of the digital pads which are characteristic of the males in sexual maturity. The sex of the young specimens was determined by dissection.

Preliminary results were published by PALCICH (1959): our new data have been added to these.

3. THE CHARACTER "YELLOW STRIPES" OF THE VAR. "VITTATA"

The individuals of this variety (*vittata*) are characterized by the fact that they have on the upper surface of the body 3 longitudinal yellow stripes: one of which runs medially, the two others laterally; the median extends from the tip of the snout to the cloacal aperture. The stripes are broad and contrast against the blackish coloration of the ground: in some specimens the stripes are very bright.

From the beginning of our investigation we were struck by the fact that the individuals of the var. *vittata* are very difficult to find. With the hope of finding an area populated almost entirely by individuals with yellow-stripes, we made an accurate search in different areas, but everywhere we found that yellow striped individuals occur together with those with black spots. It was also found that the black spotted specimens are more frequent.

In table I the relative frequencies of individuals of the two varieties, captured in the district of Palermo in several years, are reported: as shown the individuals of the var. *vittata* constitute only 6,9% of the population.

TAB. I.

3250 adults of Discoglossus pictus collected in the district of Palermo distributed according to their coloured pattern.

Var.	Number ind.	%
<i>ocellata</i>	3029	93,1
<i>vittata</i>	226	6,9

A similar investigation carried out in different areas in Sicily gave the results reported in table II: they are not very different.

In table III 2703 animals were distributed according to their coloration and sex. The data show that neither coloration is limited to the sex: males and females are present in each variety. The table shows also that in both varieties the males are more

frequent than the females; this point will be investigated more fully in the future.

TAB. II.

389 adults of Discoglossus pictus collected in different districts of Sicily distributed according to their coloured pattern.

District	Total	Var. ocellata		Var. vittata	
		n.	%	n.	%
Messina	85	79	92,9	6	6,1
Catania	56	54	96,4	2	3,6
Agrigento	84	81	96,4	3	3,6
Siracusa	22	21	95,4	1	4,6
Caltanissetta	34	32	94,1	2	5,9
Trapani and Mazara .	77	75	97,4	2	2,6
Regalbuto	31	27	87,1	4	12,9
Totals		369	94,8	20	5,2

TAB. III.

2703 adults of Discoglossus pictus distributed for sex and coloured pattern.

Variety	Number ind.	♀♀	♂♂	P for sex-ratio 50 %
<i>ocellata</i>	2489	1168	1321	0.01
<i>vittata</i>	214	88	126	0.01
Totals	2703	1256	1447	

For the equal distribution of the sexes in the two varieties:
 $X^2 = 2.65$
 $n = 1$
 $P = \pm 0.2$

4. THE CHARACTER "BLACK SPOTS" OF THE VAR. OCELLATA.

The "black spotted" specimens are characterized by numerous black spots on the back: the shapes and the sizes of the spots are variable; the shape is usually oval; the spots are generally separate

except in the interscapular region where they join to form a sort of X; the shape of this X varies considerably, depending upon the number and the shape of the constituent spots: generally 8 to 10 spots are involved.

Behind the X, one can notice a distinct yellow spot: its shape is triangular or trapezoidal; in some individuals, however, it is missing.

The other spots are arranged in 4 longitudinal stripes: the pattern, however, varies. In the median posterior region of the back one can also notice, in some individuals, an inconspicuous yellow stripe. In some individuals the black spots are large and with a yellow border; males and females do not show any difference for this pattern.

5. RESULTS OF THE CROSSES

Crosses are very easy to do in the laboratory. The selected specimens are put together in an aquarium, their eggs isolated and the tadpoles raised until metamorphosis. The coloured pattern appears just before metamorphosis.

The following crosses were performed:

a) Cross: "*black spotted*" X "*black spotted*"

The results from 16 crosses are reported in table IV. As shown clearly in the table, from such crosses one obtains only "*black spotted*" individuals.

TAB. IV.

Results from 16 crosses between individuals
"*black spotted*" × "*black spotted*"

Number of individuals	"black spotted"	"yellow striped"
1719	1719	—

b) Cross: "*yellow striped*" X "*yellow striped*"

The results of 16 crosses are reported in tab. V. From these crosses were obtained "*yellow striped*" and "*black spotted*"

individuals; only in exp. 1 (and possibly in one of the exp. 8-10) 100% of "yellow striped" individuals were obtained. If the data of exp. 1 and 8-10 are not calculated it results that the "yellow striped" specimens are 75% and the "black spotted" 25%.

TAB. V.

*Results from 16 crosses between individuals
"yellow striped" × "yellow striped"*

Experiments	Total of indiv.	"yellow striped"	"black spotted"
1	14	14	—
2	27	20	7
3	23	16	7
4	60	48	12
5	22	14	8
6	40	32	8
7	106	81	25
8-10	907	734	173
11	70	25	45
12	51	35	16
13	64	57	7
14	61	41	20
15	88	69	19
16	138	124	14

Total = 1671 "yellow striped" = 1310 (78.6%) (ratio 3:1) $\chi^2 = 10.25$
 "black spotted" = 361 (21.4%) $P = 0.001$

In exp. 8-10 three couples of "yellow striped" specimens were put together: probably one of these couples gave only "yellow striped" individuals. If exp. 1 and 8-10 are not considered the results of the crosses are: 75% "yellow striped" and 25% for "black spotted", as in a Mendelian cross between two F_1 heterozygotes.

c) Cross: "yellow striped" X "black spotted"

In the experiments of this type the character "yellow striped" was sometimes brought by the male, sometimes by the female: the result, was, however, always the same, as reported in tab. VI. One can see that 50% of the individuals result "yellow striped" and 50% "black spotted".

From the above one can conclude that the coloured pattern is dependent on an autosomal gene; and that the gene for "yellow striped" is dominant to the gene for "black spotted". Indicating the alleles as V (= vittata) and v, the constitution of the "yellow striped" individuals is VV or Vv, and that of the "black spotted" individuals is vv.

TAB. VI.

Results from 10 crosses between individuals
 "yellow striped" \times "black spotted"

Cross	Total	Yellow striped	Black spotted
a) yellow ♂ \times ♀ spotted	86	42	44
" ♂ \times ♀ "	39	23	16
" ♂ \times ♀ "	12	7	5
" ♂ \times ♀ "	124	67	57
" ♂ \times ♀ "	148	68	80
" ♂ \times ♀ "	201	102	99
b) yellow ♀ \times ♂ spotted	6	4	2
" ♀ \times ♂ "	147	80	67
" ♀ \times ♂ "	184	107	77
(not determined)	247	120	127
	1194	620	574

$\times \times$ For ratio 50% = $X^2 = 1.772$
 $n = 1$
 $P = 10.2$

6. THE COLOURED PATTERN IN SPECIMENS SHORTLY AFTER METAMORPHOSIS

The results of the crosses presented above show that the coloured pattern of the individuals of the two varieties of *D. pictus*, is determined by an autosomic gene; and that the allele for "yellow stripes" is *dominant* on that for "black spotted".

Does the last conclusion agree with the fact that "yellow striped" individuals are only 6.9% of the population?

As the noticed frequency refers to adult specimens we proposed to check its validity in recently (5-6 weeks) metamorphosed specimens: so we collected 1647 small *Discoglossus* in the field and we distributed them according to their colour. The results, presented in table VII, show that the percent of "yellow striped" individuals is about the same as that noticed for the adults.

To imitate the conditions met in nature the following experiment was devised in the laboratory.

TAB. VII.

Very young (metamorphosed since some weeks) Discoglossus pictus distributed according to their coloured pattern.

Total	" Black spotted "		" Yellow striped "	
	Total	%	Total	%
1647	1559	94,6	88	5,4

TAB. VIII.

	Black spotted	Yellow striped
Beginning of experiment	883	569
End of experiment	28 (25,02)	13 (15,93)

In a small terrarium, containing sufficient nourishment (*Tubifex*) and also 4-5 " medium-size " predatory specimens of *Discoglossus*, (which usually eat the small ones) 883 " black spotted " and 569 " yellow striped " specimens were introduced: the specimens were placed in the terrarium as soon as they metamorphosed. After about 3 months the remaining individuals were counted; one obtained the following result: 28 " black spotted " and 13 " yellow striped ". The result is reported in Tab. VIII.

7. DISCUSSION

1. Our investigation on the relative frequency of the individuals of the var. *vittata* with respect to those of the var. *ocellata* leads to the affirmation that the coloured pattern, characteristic for these two varieties, is controlled by an autosomic couple of genes and that the gene for " yellow stripes " is dominant on that for " black spots ".

Our results agree with those of LANTZ (1947), GALLIEN (1948), BRUCE and PARKES (1947), ALONSO-BEDATE (1960) but not with those of KNOEPFFLER (1962). This last author obtained 41 metamorphosed specimens from the cross *D. pictus* ♂ × *D. sardus* ♀: they all presented the "black spotted" pattern. "Tous les animaux—according to the author—obtenus par croisement sont rigoureusement identiques entre eux de forme et de dessin. De plus, aucun des hybrides n'arbore la livrée rayée de la forme *pictus*... Que conclure de ce fait? Les rayures dorsales de *D. pictus* sont un caractère récessif qui disparaît au moment du croisement." The author explains the results of LANTZ and GALLIEN, admitting that the specimens used in their crosses, in fact, did not belong to *sardus* but to *pictus* of var. *ocellata*: "Comment LANTZ et GALLIEN ont-ils obtenus des hybrides rayés? Il semble que les *D. pictus sardus* employés par ces auteurs aient été en réalité des *D. pictus* de la forme ocellée". From this quotation it seems that in Sardinia there would be specimens of both species, *pictus* (var. *ocellata*) and *sardus*.

2. The situation of *Discoglossus* is not new, in the anurans.

In *Rana pipiens* two varieties have been also described: the "wild" type, with the characteristic spotting pattern (due to gene, which, however, operates with an integrating system of modifiers) and the "burnsi" type due to a mutation suppressing the formation of the black spots on the back (VOLPE 1961; MOORE 1942).

In *Bombina* also two varieties have been described, one with a yellow abdomen (found in Italy, France etc.) and another with a red abdomen (Denmark, Russia etc.). The colour would also be in this case dependent on a pair of genes, and the gene responsible for "yellow" would dominate over that responsible for "red" (cfr. CUÉNOT 1936, p. 155).

3. Results deriving from transplanting fragments of the neural crests from specimens of one variety on specimens of the other variety (ALONSO-BEDATE 1959; LA SPINA 1963) show how independent are the genes responsible for the coloured pattern. The realization of the original coloured pattern is not disturbed by the genotype of the host with which the transplant enters in relation: chromatophores VV or Vv always give rise to the typical "yellow striped" pattern when transplanted on a host whose genetical

constitution is vv. Vice versa, chromatophores vv always give rise to the typical "black spotted" pattern, when transplanted on specimens whose genetic constitution is VV or Vv. Offering to chromatophores vv a VV or Vv mesoderm (or entomesoderm) they still give rise to the "black spotted" pattern; a "yellow striped" pattern is also obtained when VV or Vv chromatophores are compelled to migrate on vv mesoderm (or mesentoderm).

4. As shown, the gene for "yellow striped" is *dominant* to the gene responsible for the "black spotted" pattern; although the gene is dominant, the relative frequency of the "yellow striped" individuals is very low in nature. We are not able to give an explanation of this fact; probably the explanation is much more complex than one can suppose.

ACKNOWLEDGEMENT

The author wishes to thank Prof. MONTALENTI and Dr. CARFAGNA for the statistical elaboration of the data and for their kind advice.

ZUSAMMENFASSUNG

In Sizilien tritt *Discoglossus pictus* in zwei Varietäten, nämlich var. *vittata* und var. *ocellata* auf. Ihre Verbreitung scheint sich auf ganz Sizilien zu erstrecken; var. *ocellata* ist bedeutend häufiger (94%) als var. *vittata* (ca. 6%). Aus Kreuzungsversuchen zwischen Individuen der gleichen bzw. der beiden Varietäten geht hervor, dass das Zeichnungsmuster durch ein autosomales Genpaar bedingt ist. Das Gen für das Merkmal "gelb gestreift" ist dominant über das Gen für das Merkmal "schwarz gefleckt". Für "gelb gestreifte" Individuen gilt demnach der Genotypus VV bzw Vv, für "schwarz gestreifte" hingegen vv. Die Ursache für die geringe Häufigkeit der "gelb gestreiften" Individuen ist nicht bekannt.

RÉSUMÉ

En Sicile on rencontre deux variétés de *Discoglossus pictus*, à savoir les variétés *vittata* et *ocellata*. Les deux variétés sont répandues sur toute la Sicile, la variété *ocellata* étant toutefois

plus fréquente (94%) que *vittata* (env. 6%). Des croisements entre individus de la même ou des deux variétés démontrent que la coloration est déterminée par une paire de gènes autosomiaux. Le facteur "raies jaunes" est dominant sur le gène "taches noires". Les individus à raies jaunes ont donc le génotype VV ou Vv tandis que ceux à taches noires sont du type vv. La raison pour laquelle les individus à taches jaunes sont rares n'est pas connue.

SUMMARY

In Sicily there are two varieties of *Discoglossus pictus*: the var. *vittata* and the var. *ocellata*. They seem to be represented all over in Sicily. Specimens of the var. *ocellata* are more frequent (about 94%) than those of *vittata* (about 6%).

From crosses between individuals of the same or of different variety it results that the colour pattern depends on a pair of autosomic genes; and that the gene responsible for the "yellow striped" pattern is *dominant* to the gene responsible for the "black spotted" character. The "yellow striped" specimens can be indicated as VV or Vv; the "black spotted" specimens vv. The possible cause responsible for such a low proportion of "yellow striped" specimens in the population is not known.

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Reverberi, C. 1964. "Data concerning the relative distribution of two varieties of *Discoglossus pictus* in Sicily (Amphibia)." *Revue suisse de zoologie* 71, 203–214. <https://doi.org/10.5962/bhl.part.75606>.

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