After LSD[9], the observed decrease in mean EEG integrated values (proportional to the standard-deviation of EEG over the 20 sec integration epoch) and in variability coefficient computed between integrated values (inversely proportional to the signal-to-noise ratio[10]) confirms the present findings of signal-to-noise increase. This would indicate not only an increase in signal effects, especially during contralateral stimulation, but also a decrease in background EEG activity taken as a noise indicator.

[9] L. GOLDSTEIN and R. A. BECK, Int. Rev. Neurobiol. *8*, 265 (1965).
[10] P. R. ETEVENON, G. GUILLON, J. R. BOISSIER, Agressologie, *10*, 641 (1969).

Résumé. L'administration de LSD augmente les effets de la stimulation contralatérale, la latéralisation et le rapport signal-sur-bruit. Ces résultats sont en accord avec le modèle de fonctionnement cérébral considéré comme un détecteur cohérent en parallèle.

P. ETEVENON and J. R. BOISSIER

Unité de Recherche de Neuropsychopharmacologie, INSERM
2, rue d'Alésia,
F-75 Paris 14e (France),
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The Role of Melanoblasts in Melanophore Pattern Polymorphism of *Xiphophorus* (Pisces, Poeciliidae)

Species and species hybrids in the genus *Xiphophorus* (which includes *Platypoecilus*) may develop pigment cell patterns, which consist of extremely varied numbers of macromelanophores. These can be correlated to the genetic constitution of an animal[1,2]. Since melanophores[3,4] and melanocytes[4] are rarely, if ever, observed in division stages, one must suppose that genes influencing melanophore numbers act at an earlier level of pigment cell differentiation. Therefore, melanoblast[4] densities in adult animals of diverse genotypes[5] from pure species and from species crosses have been studied. For practical reasons, counts were restricted to melanoblasts which are attached to scales. Because of the heterogeneity of distribution, only maxima[6] and minima[6] are given in the Table.

The relationship between genotypes and melanoblast densities is considered based at first on the maximal values, since it has been determined that average numbers for whole animals generally approach them. Densities of about 200 melanoblasts per mm² (Figure 1) are found in members of pure species (see 1–3, 5, 6, 10, 11 in the Table) as well as in hybrids of different genotypes (see 12–14, 16–18 in the Table). The phenotypes within this group vary extremely, from animals bearing no macromelanophore spots (see 1, 3, 14, 17 in the Table) to melanoma or premelanoma bearing animals (see 12, 13 in the Table). From these counts there seems to be no correlation between the number of detectable melanoblasts and the melanophore promoting constitution of a genotype, the degree of which is indicated by more or less extended melanophore concentrations.

Additional results must be taken into consideration for cases of lower maxima (see 4, 7–9, 15 in the Table), which seem not to agree with this general conclusion. Actually, all numbers given have to be regarded as minimal counts only.

[1] Review F. ANDERS, Experientia *23*, 1 (1967).
[2] C. D. ZANDER, Mitt. Hamburg. Zool. Mus. Inst. *66*, 241 (1969).
[3] Discrimination between micro- and macromelanophores is not necessary, when used in this context. See C. BECKER-CARUS, Sber. Ges. naturf. Freunde Berl., N. F. *5*, 136 (1965).
[4] Nomenclature from A. LEVENE, V. J. MCGOVERN, Y. MISHIMA and A. G. OETTLE, in *Structure and Control of the Melanocyte* (Ed. G. DELLA PORTA and O. MÜHLBOCK; Springer-Verlag, Berlin, Heidelberg, New York 1966), p. 1.
[5] We are greatly indebted to Miss K. KLINKE for providing us with fish specimens.
[6] Maxima are normally found in the ventral ridge, the back beneath the dorsal fin and the throat. Minima often occur in the region of the middle line.

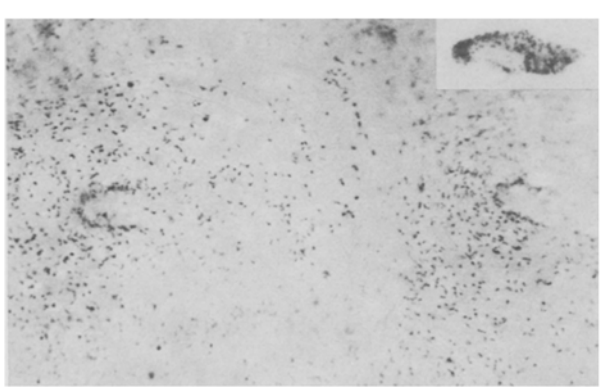

Fig. 1. Dense concentration of melanoblasts (approximately 200/mm²) on scales in situ covering the belly of an adult *X. helleri*/*maculatus* hybrid. Each small dot represents one melanoblast (see insert), except in single cases of very small melanophores. (The whole fish was fixed[11] at 4 °C for 2 h and rinsed thoroughly overnight. Dopa incubation[11] was done for 5–6 h with one change of solution after 1–2 h). × 20; insert × 650.

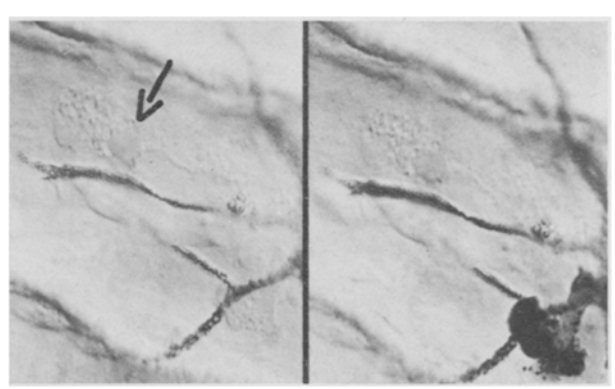

Fig. 2. Part of a scale of *X. helleri* before (left) and after Dopa incubation (right). 2 melanoblasts have been stained, 2 other ones (arrow) have remained colourless. Interference contrast, × 900.

Approximate melanoblast densities on scales[7] in adult *Xiphophorus*, based on at least 4 specimens and 20 scales from each

No.	Species or hybrid[8]	Macromelanophore Gene[8]	Melanoblasts per 1 mm² Maximum[6]	Minimum[6]
1	*X. helleri* (Rio Lancetilla)	*–/–*	250	100
2	*X. helleri* (Rio Lancetilla)	*Sp/–*	200	80
3	*X. montezumae*	no manifestation	150	65
4	*X. montezumae*	*Sc/?*	100	40
5	*X. variatus*	*Sr/Sr*	170	70
6	*X. variatus*	*Sr/P*	200	100
7	*X. xiphidium*	*–/–*	80	10
8	*X. maculatus*	*Sd/Sd*	80	40
9	*X. maculatus*	*Sp/Sp*	100	10
10	*X. maculatus*	*Sd/Sr*	180	10
11	*X. maculatus*	*N/N*	180	10
12	F_1 (*X. mac./hell.*)	*Sd/–*	250	100
13	1st backcross (*X. mac., hell./hell.*)	*Sd/–*	200	70
14	1st backcross (*X. mac., hell./hell.*)	*–/–*	185	40
15	2nd backcross (*X. mac., hell./hell.*)	*Sd/–*	80	20
16	*Albino*[9], nth backcross to *X hell.*	*Sd/–*	300	100
17	*Albino*[9], nth backcross to *X hell.*	*–/–*	300	130
18	*Golden*[10] backcross to *hell.*	*–/–*	350	100

This is, first of all, demonstrated by melanoblasts, which do not stain with dopa, but are detected in interference contrast (Figure 2). A second observation was made in regeneration experiments, in which skin was removed to the muscle layer. In all animals tested, which belonged to very different genotypes, melanoblasts were detected only 5 days later in concentrations of 300 to 800 per mm² in regenerating parts. Since division stages were not observed and diminuition of melanoblast numbers in peripheral regions of the fishes did not occur, melanoblasts in regeneration tissues have to be regarded as being truly additional to those which are normally present in the skin. Finally, it must be pointed out that only the majority of peripheral melanoblasts is located on the scales.

These three observations lead to the conclusion that the actual number of melanoblasts in a given body region is a minimum, the upper limit of which cannot be estimated. This means that the approximate average number of 200 per mm² for disposal has not at all been exaggerated.

Its significance becomes obvious when the fact is considered that a melanoblast can differentiate to a macromelanophore of about 400 µm in diameter. If all 200 melanoblasts in 1 mm² would do this, then they would be arranged in a layer 25 melanophores thick in that limited area, thus causing not only a totally black body region, but even a compact pigment cell mass. That means that the number of melanophore precursors can be regarded as being large enough to permit melanophore formation to almost any degree of melanophore spot extension. Even melanoma development could occur, inspite of the fact that differentiation of pigment cells is usually performed only to the melanocyte stage[9].

All this indicates that genes influencing the melanophore pattern do not act on the number of reservoir melanoblasts. Instead they must determine the proportion of melanoblasts which continue their differentiation to pigment cells. If this gene activity were restricted to a specific body region, pattern formation would result from that action of macromelanophore genes which would allow melanophore differentiation out of a rather abundant reservoir of precursors. Therefore there seems to be no need to hypothesize specific genes or distinct parts of a more complex genetic unit for melanophore number other than one which determines melanophore distribution[12, 13].

Zusammenfassung. Bei *Xiphophorus*-Arten und -Artbastarden wurden mit Hilfe der Dopa-Reaktion Anzahl und Verteilung von Melanoblasten untersucht. Bei Adulten wurden, unabhängig vom Genotyp, in der Haut sehr viele Melanoblasten (200/mm² und mehr) nachgewiesen, darüber hinaus zeigten Regenerationsversuche, dass dieses Reservoir noch vermehrt werden kann. Danach wird angenommen, dass die sogenannten Makromelanophorengene keinen Einfluss auf die Zahl der Melanophorenvorläufer haben, sondern ihre Wirkung ausüben, indem sie mitbestimmen, wieviele der stets vorhandenen sich weiterdifferenzieren.

W. Lueken and U. Kaeser

Genetisches Institut der Justus Liebig-Universität, D-63 Giessen (Germany), 27 April 1972.

[7] This refers only to the distal part, which is ordinarily the only part to bear pigment cells.

[8] For origin of stocks and gene nomenclature see[2] and K. D. Kallman and J. W. Atz, Zoologica, N. Y. *51*, 107 (1966).

[9] J. Vielkind, U. Vielkind and F. Anders, Cancer Res. *31*, 868 (1971).

[10] Phenotype characterized by reduction of melanophore numbers, formerly referred to as 'stippled'. See F. Anders, K. Klinke and U. Vielkind, Biol. in unserer Zeit *2*, 35 (1972).

[11] Y. Mishima, J. invest. Dermat. *34*, 355 (1960).

[12] This research was accomplished with the aid of the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

[13] We thank Mr. K. Lepper (Giessen) very much for valuable technical assistance and Miss D. Pursglove (Giessen) for critical reading of the manuscript.