2 rotting saguaros about 70 m apart were selected for the present experiment. In one of them, about 3200 flies collected on 21 November 1974 were marked with micronized red powder, fluorescent to UV-light (Helecon Fluorescent Pigments, US Radio Corporation), and released at the same place on the same day. Similarly, in the other cactus about 6200 flies collected simultaneously, were marked green and released. In the 2 following days a total of 274 *D. nigrospiracula* mating pairs were collected and scored by colour. This makes it possible to distinguish 2 kinds of flies in each site according to their colour. Flies found marked with the same colour used at the site of collection will be considered nonmigrant flies or residents. On the other hand, flies of different colour will be migrants. At the same time, a random sample of flies was taken each day at each site by sweeping a net over it. The results of these collections on 2 consecutive days are shown in the table.

The distributions of unmarked flies for mating and random samples were compared (table) to detect any possible effect of marking on the mating behavior of the flies. A χ^2-test for homogeneity (with Yates' correction for continuity) at both sites gave nonsignificant results and indicates that we can take the marked sample of flies as representative of the whole population at each site. Most interesting is the distribution of migrants compared to the distribution of residents or nonmigrant individuals. At the green site, migrant individuals mate more frequently than resident individuals. The χ^2-test for homogeneity (with Yates' correction for continuity) is significant ($p < 0.05$) at the green site. However, no significant deviation from homogeneity was found at the red site.

In order to account for the low figures of the table, we have also performed other statistical tests less conservative than χ^2, namely G-test and Fisher's exact test of independence. The results are virtually the same. With G-test, the significance at the green site remains high ($0.05 < p < 0.10$) and Fisher's exact test gives a probability of $p = 0.031$. We do not know at the moment the actual causes of this mating advantage of migrants under certain circumstances, but we know that a minority mating advantage has been found among populations and strains in several cases[7]. In the last column of the table, we have computed the ratio of migrants to residents for both mating and random individuals at each site. It is clear that relative low numbers of migrants in the random population are associated with high relative numbers of migrants which actually mate. On the other hand, migrants seem to lose their mating advantage when they are more abundant in the population. This suggests a type of frequency-dependent selection associated with migration which will increase the genetic fitness of migrants when migration rate is very low. The consequences of this peculiar mating behavior would be to increase the gene flow values at low migration rates, and it will bring about a higher than expected tendency to genetic homogeneity among populations which exchange very low numbers of individuals.

We do not know at the moment whether the actual causes of this mating advantage under low migration rates are due to a true higher mating activity of migrant individuals with a genetic component or to a special recognition system among subpopulations. Migrant selection has been cited as a means of maintaining genetic polymorphism[8] and has been documented in *Microtus*[9]. However, its existence in *Drosophila* has only been inferred from some laboratory studies[10]. The present work is the first direct evidence[11] of migrant selection operating under natural conditions.

Distribution of numbers of marked and unmarked flies in mating and random samples collected at 2 natural breeding sites of *Drosophila nigrospiracula*

	Unmarked	Marked			
	Total	Migrants (M)	Residents (R)	Total	Ratio (M/R)
Red site					
Mating sample	332	3	29	32	0.103
Random sample	733	13	43	56	0.302
Green site					
Mating sample	174	3	7	10	0.429
Random sample	1043	2	45	47	0.044

7 C. Petit and L. Ehrman, Bull. Biol. *4*, 433 (1968).
8 E. O. Wilson, Sociobiology. Harvard University Press, Boston 1975.
9 J. H. Myers and C. J. Krebs, Ecol. Monogr. *41*, 53 (1971).
10 T. Narisse, Evolution *22*, 301 (1969).
11 J. S. Johnston, working on the same species, has recently confirmed that his results also suggest that frequency of mating in migrants is significantly higher than migration rate.

Evidence that chromatophores of cephalopods are linked by their muscles[1]

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Summary. Light microscopy of living skin and electron microscopy confirm the hypothesis that chromatophores of cephalopods are linked horizontally by their muscles. Earlier physiological evidence is discussed and interpreted.

The structure and function of the cephalopod chromatophore organ is well understood: expansion of the pigment cell is mediated by a set of radial muscles and contraction by the action of a cytoelastic sac which is located inside the chromatophore cell[4–7].

Compared to our knowledge of individual chromatophores, little information is available concerning the spatial organization of an ensemble of chromatophores, especially the morphology of their muscles[4].

It was once believed that the chromatophore muscles form a syncytial system surrounding the chromatophore cell, and that the distal ends of the radial fibres are attached to either strands of connective tissue[8] or skin muscles[9]. Bozler has produced convincing evidence against the syncytial nature of the chromatophore muscles. He demonstrated electrophysiologically that each radial fibre is an individual cell, functioning independently of the adjacent ones[10].

The current view of chromatophore muscles is essentially that of Bozler, i.e. the radial muscle fibres are single, unbranched cells, with the nucleus at the periphery of the chromatophore and the distal end some distance away in the connective tissue.

In the present paper we produce light and electron microscopic evidence that chromatophore muscles do not end blindly (as suggested to Bozler), but interconnect individual chromatophores.

Materials and methods. Biopsies of skin of about 1 cm² were obtained from anaesthetized animals. The samples were pinned on a wax plate, surface down, and carefully cleared of the subepidermal connective tissue under the microscope. The skin was then placed on a slide, again surface down, and covered with a cover slip. This preparation was done with skin of 6 species (*Loligo vulgaris, Sepia officinalis, Octopus vulgaris, Octopus salutii, Eledone cirrosa* and *Eledone moschata*) and observed immediately without further treatment on a Leitz Orthoplan micro-

1 This paper is dedicated to Professor Adolf Portmann on the occasion of his 80th anniversary.
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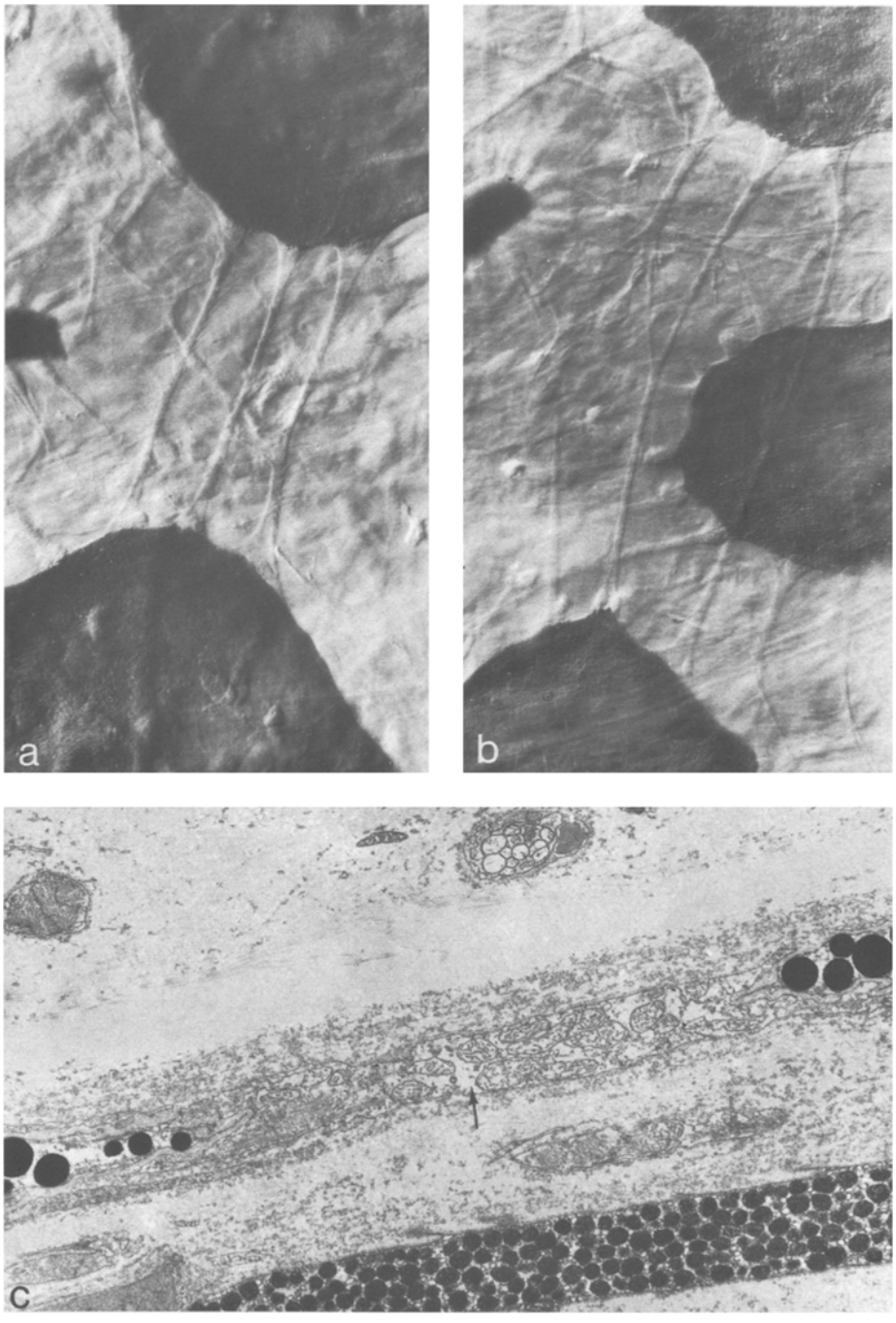
3 We wish to thank Prof. J. Z. Young and Dr P. R. Boyle for helpful discussion and the Swiss Government for financial support.
4 E. Florey, Comp. Biochem. Physiol. *18*, 305 (1966).
5 R. A. Cloney and E. Florey, Z. Zellforsch. *89*, 250 (1968).
6 W. Weber, Z. Zellforsch. *108*, 446 (1970).
7 S. Mirow, Z. Zellforsch. *125*, 176 (1972).
8 P. Girod, Archs Zool. exp. gén. *1*, 225 (1883).
9 F. B. Hofmann, Pflügers Arch. ges. Physiol. *118*, 375 (1907).
10 E. Bozler, Z. vergl. Physiol. *7*, 379 (1928).



a, b 2 interference contrast micrographs of unstained skin of *Eledone cirrosa*. Muscles run between the chromatophores. ×750.
c Electron micrograph of a muscle running between 2 brown chromatophores (arrow). A yellow chromatophore (with pigment granules about half the size of the brown ones) is shown underneath. ×4500.

scope equipped with interference contrast optics. For electron microscopy, small slices of skin were immersed in 2% osmic acid dissolved in a cacodylate-buffered Ringer of 1180 mosm. Thin sections were photographed on a Philips 301.

Results. In isolated skin there is a considerable measure of spontaneous activity of chromatophores: phasic and tonic expansion as well as activity progressively spreading over a distance can be observed up to several hours after the removal of skin samples. This phenomenon offers an excellent possibility for the study of the organization and functioning of chromatophore organs.

The chromatophores are arranged in 4 or 5 different layers[11]. The lowest one is occupied by very small, faint yellow chromatophores, which have an incomplete set of muscle or none at all; we suppose that these are the youngest members of a population with continuous proliferation and loss, because we regularly find degenerating chromatophores in the top layer[12]. Spontaneous activity of chromatophores in isolated skin, and most probably all activity in intact skin, is restricted to the 2 or 3 layers between the presumed germ layer and the degenerating layer. As can be observed under the microscope, discrete groups of chromatophores expand and contract synchronously or almost synchronously while others remain silent.

It was not found, until we used interference microscopy, that those chromatophores, which operate synchronously or nearly synchronously, are all interconnected horizontally by their muscles (figure); frequently the muscles split into 2 or more branches. It is difficult to determine the dimension of individual muscles, but during their contraction it can be seen that they terminate either on other chromatophores or on other chromatophore muscles. They can form elaborate patterns of connectivity between several chromatophores.

Although we were unable to determine all the connections of any particular chromatophore, we estimate that each one is connected directly with 8–14 other chromatophores, perhaps even more. There is apparently no vertical connection between chromatophores of different layers. All species investigated show chromatophore link.

Discussion. The finding that chromatophores in cephalopods are connected by muscles, does not fundamentally conflict with earlier physiological evidence; however, in some points the picture must be revised:

a) Although the chromatophore muscle is an independent functional entity[4,5,10], chromatophores cannot be operated individually, because they are linked. As one can observe directly under the microscope, the full expansion of 1 chromatophore involves automatically the partial expansion of approximately 10 neighbouring chromatophores.

b) Spontaneous progressive spread of chromatophore expansion in isolated skin (or dying or denervated skin[9,13]) can now be reasonably explained: in living skin there is a physiological barrier to prevent excitation transfer from one muscle to the other[4]. Post mortem, the membrane resistance is lowered and excitation can cross at myomuscular junctions, travelling along the muscular network over considerable areas. (This phenomenon was named 'Wolkenwandern' or wandering clouds by Hofmann.)

c) As suggested by Packard[14], the consistency of colour patterns in *Octopus* may be due to the patterned distribution of chromatophore nerves. However, it cannot be ruled out now that muscular link as well can be a basis for the consistency of colour patterns in cephalopods.

11 D. Froesch, in preparation.
12 D. Froesch and J. B. Messenger, in preparation.
13 G. D. Sanders and J. Z. Young, Proc. r. Soc. Lond. *B 186*, (1974).
14 A. Packard, J. Physiol. *238*, 38 (1974).

Métaplasie épidermoïde de l'épithélium mammaire humain en culture organotypique à long-terme

Squamous metaplasia of human mammary epithelium in long-term organ culture

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Summary. Squamous metaplasia of the mammary epithelium was observed in human breast tissue maintained in long-term organ culture. The phenomenon occurred only in the synthetic medium 199 with Earle's salts. Insulin and/or glucose enrichment enhanced its occurrence.

L'apparition de phénomènes de métaplasie squameuse ou malpighienne dans l'épithélium mammaire maintenu en culture organotypique a été rapportée dans différentes observations[1–5]. En ce qui concerne la glande mammaire humaine les conditions dans lesquelles apparait la métaplasie n'a pas été étudiée. Au cours d'un travail portant sur le maintien en survie à long-terme et l'influence d'additifs hormonaux sur la glande mammaire humaine adulte normale et au repos nous avons observé ces phénomènes dans des conditions de culture précises. Le but de cette communication est de présenter les facteurs physico-chimiques qui interviennent dans la genèse de la métaplasie malpighienne dans la glande mammaire humaine explantée.

Méthodes. Le matériel d'étude a consisté en du tissu mammaire provenant de 13 femmes (âge moyen: 30,5 ans) soumises à une intervention de correction chirurgicale. Nous avons cultivé un total de 5000 explants par immersion dans le milieu synthétique 199, que nous avons utilisé dans les formules de Earle et de Hanks. La diffé-

1 F. L. Archer, Archs Path. *85*, 62 (1968).
2 B. E. Barker, H. Fanger et P. Farnes, Expl Cell Res. *35*, 437 (1964).
3 B. A. Flaxman et E. J. Van Scott, Cancer Res. *32*, 2407 (1972).
4 B. A. Flaxman, J. Invest. Dermat. *61*, 67 (1973).
5 H. Koyama, D. Sinha et T. Dao, J. nat. Cancer Inst. *48*, 1671 (1972).