

ously been known to possess desiccation-tolerant mature foliage; *Oropetium capense* Stapf (see Table 1), *Sporobolus stapfianus* Gand., *Eragrostis denudata* Hack. ex Schinz, and *Microchloa caffra* Nees were found to endure dehydration to air dryness in the laboratory, that is, approximately 30 to 40 percent relative humidity. In addition, the desiccation-tolerant sedge *Ficinia filiformis* Schrad. was clearly palatable to grazing animals. Unfortunately, time and scarcity of material did not allow a full investigation of these species.

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Melanin Pigmentation: An in vivo Model for Studies of Melanosome Kinetics within Keratinocytes

Abstract. *The phagocytosis of latex beads by epidermal cells is proposed as a model for studies on melanosome kinetics within the epidermis. Large latex beads (0.8 micrometer) are ingested singly, whereas small beads (0.1 micrometer) are taken up in groups, results showing that the uptake mechanism depends on the size of the individual particles. This size-dependency may explain the different distribution patterns of melanosomes and thus the differences of skin color in the Caucoid and Negroid races.*

Most of the knowledge on mechanisms of pigment transfer and on the fate of melanosomes within epidermal cells is derived from experiments with tissue culture (1). Studies of pigmented

tissues with the electron microscope appear to confirm these data (2), but there is still little information on the dynamics of these phenomena in vivo. Since the uptake of melanosomes by

keratinocytes is a heterophagic process (3), the phagocytosis by epidermal cells of particles resembling melanosomes might prove a useful model for studies on melanosome kinetics within keratinocytes.

Polystyrene latex beads (4) are suitable for this purpose because their shape resembles that of melanosomes and their sizes can be adjusted to those of melanosomes. Subepidermal blisters were produced in guinea pigs with a suction blister device (5), and suspensions of latex beads were injected percutaneously into the blisters. This provided direct contact between the latex particles and the epithelial cells that, in the healing phase, advanced over the denuded floor of the blisters. Examination with the electron microscope revealed that the latex beads were avidly ingested by the keratinocytes and were incorporated into phagosomes limited by single membranes (Figs. 1 and 2). The mode of uptake and of intracellular transport of the particles and their inclusion into the lysosomal system as shown by the presence of acid hydrolase activity inside the delimiting membrane have been described (6). Here we report that (i) uptake of the latex beads into keratinocytes occurred with ease and (ii) the mechanism involved depended on the size of the beads, since large beads (0.8 by 0.4 μm) were always ingested singly (Fig. 1) whereas small ones (0.1 by 0.05 μm) were taken up in groups (Fig. 2). This mode of uptake resembles phagocytosis of latex beads by acanthamoeba, which ingests large particles (1.305 μm) individually and small ones (0.537 μm) together as a compact mass (7).

The similarities between this model system and the melanosome system in vivo are striking. Latex beads resemble melanosomes in shape and size, both are incorporated, either singly or in groups, into phagosomes of keratinocytes, and both are exposed to the action of hydrolytic enzymes within lysosomes (6, 8). This experimental system can be manipulated at will and thus permits exploration of problems that are difficult to study directly in the melanosome system in vivo.

For example, the model system already provides a significant clue to a fascinating phenomenon of pigmentation: the strikingly different distribution patterns of melanosomes within Caucoid and Negroid keratinocytes—distribution patterns that are held responsible for the differences of skin color in the two races (9). In Negroids

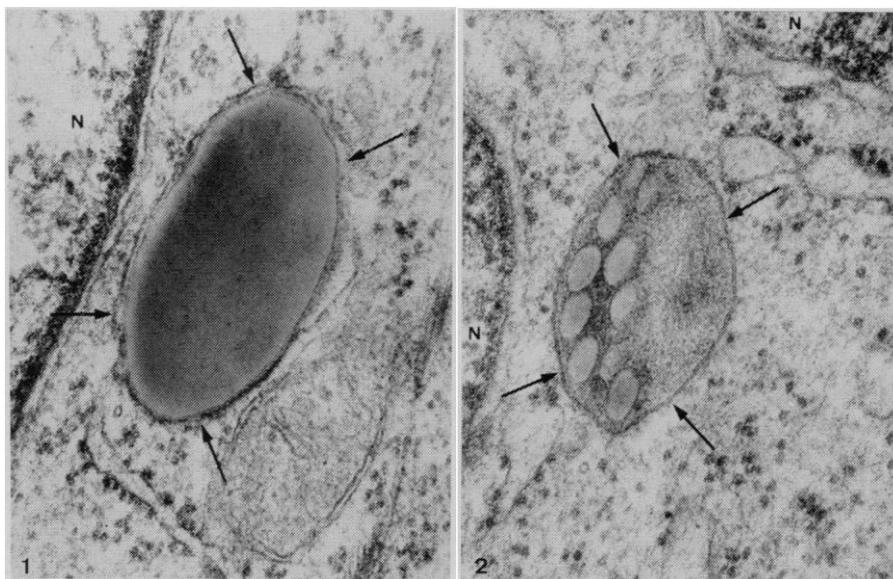


Fig. 1 (left). Large latex particles (0.8 by 0.4 μm) incorporated singly into cells. The arrows denote the delimiting single membrane of a phagosome containing a large latex bead, and N denotes the nucleus. ($\times 62,000$) Fig. 2 (right). Small latex particles (0.1 by 0.05 μm) always taken up in groups and surrounded by a common single membrane (arrows). The phagosomes are approximately equal in size to those in Fig. 1. ($\times 62,000$)

(9) and Australian aborigines (10) the melanosomes are dispersed singly within the cytoplasm of epidermal cells, whereas in Caucasoids they are packed into so-called melanosome complexes (9). Our model system demonstrates that the mode of uptake of particles resembling melanosomes is size-dependent, with the size of the individual particle determining whether particles are taken up collectively or individually, and thus whether they are packed into complexes or dispersed. We propose that the differing distribution patterns of melanosomes in the Caucasoid and Negroid races reflect differences in melanosome size, and recent observations support the validity of this proposal. Toda *et al.* (11) treated Caucasoid volunteers with Psoralen and long-wave ultraviolet light and noted that, in the exposed skin, the keratinocytes contained melanosomes dispersed singly. These melanosomes were remarkably large (1.1 by 0.6 μm) compared to melanosomes of the controls (0.6 by 0.3 μm), which were packed into melanosome complexes. Obviously, melanosomes may occur singly even in Caucasoid keratinocytes provided their dimensions exceed a certain limit. This is in agreement with the conclusions derived from the model system; by inference it may be assumed that the crucial size above which individual uptake occurs is somewhere between 0.6 and 0.8 μm .

Comparative studies of melanosome size in Negroids and Caucasoids have not been performed so far, but Szabo *et al.* (9) mention that Negroid melanosomes are "larger and wider" than those of other racial groups. From our findings we predict that exact measurements of these organelles will disclose that they are larger in Negroid than in Caucasoid skin. Accordingly, the genetically determined distribution patterns of melanosomes, and thus the skin color, in the two races would primarily depend on the melanocyte, which produces the melanosomes and determines their size, and not on the keratinocyte, which receives and packs these granules.

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Genetic Polymorphisms in Varied Environments

Abstract. *Thirteen experimental populations of Drosophila willistoni were maintained in cages, in some of which the environments were relatively constant and in others varied. After 45 weeks, the populations were assayed by gel electrophoresis for polymorphisms at 22 protein loci. The average heterozygosity per individual and the average number of alleles per locus were higher in populations maintained in heterogeneous environments than in populations in more constant environments.*

Studies by means of gel electrophoresis have disclosed a large amount of genetic variation in populations of organisms as diverse as man, drosophila flies, and oats (1-3). What mechanisms are operating to maintain this variation is an open question. According to classical population models, if all protein polymorphisms in a population were maintained by heterosis, this would lead to an unbearable genetic load (4). Some investigators have devised alternative models of population fitness which will allow many polymorphisms to be maintained by heterosis (5). Others have escaped the problem of genetic load by postulating that most protein variants are neutral to natural selection and thus neither contribute to nor lessen the fitness of a population (6). Experimental evidence has not supported the neutrality hypothesis (7).

If a form of balancing selection is maintaining these protein polymorphisms, several kinds of balancing selection are possible. There is evidence for at least two types, heterosis (8) and frequency-dependence (9). In this report evidence is presented for a multiple niche polymorphism (10, 11).

Thirteen population cages were started each with 500 *Drosophila willistoni* from a collection made at Mirasol, Brazil. This collection consisted of more than 500 single female lines that were combined to begin the cages.

Table 1 indicates the conditions in each cage. The cages used were those described by Ayala (12) in which eight

food cups are available to the flies. In Table 1, y_1 means all eight food cups were heavily seeded with bakers' yeast (Fleischmann's), y_2 means that all eight food cups were heavily seeded with brewers' yeast (Budweiser), while y_1/y_2 means that four cups were seeded with bakers' and four with brewers' yeast. Similarly f_1 (Carolina instant

Table 1. Genetic variability in populations of *Drosophila willistoni* that were maintained in laboratory cages under differing conditions. In cages 1, 2, 3, 6, and 9, only one type of yeast, medium, and temperature was present. In cages 4, 5, 7, 8, 10, and 11, one factor was varied. In cages 12 and 13, all three factors were varied. Symbols are explained in the text.

Cage	Condi- tions	Heterozy- gosity per individual (% \pm S.D.)	Alleles per locus (No.)
<i>Constant environment</i>			
1	y_1f_125	7.29 \pm .83	1.68
2	y_1f_125	7.80 \pm .82	1.68
3	y_2f_125	8.04 \pm .81	1.68
6	y_1f_225	7.96 \pm .85	1.68
9	y_1f_119	7.98 \pm .84	1.64
<i>One variable</i>			
4	y_1/y_2f_125	11.09 \pm .89	1.91
5	y_1/y_2f_125	9.06 \pm .84	2.00
7	y_1f_1/f_225	10.16 \pm .88	2.05
8	y_1f_1/f_225	9.33 \pm .88	1.91
10	$y_1f_119/25$	8.86 \pm .80	1.95
11	$y_1f_119/25$	9.21 \pm .88	1.68
<i>Three variables</i>			
12	$y_1/y_2f_1/f_219/25$	13.90 \pm .98	2.00
13	$y_1/y_2f_1/f_219/25$	12.81 \pm .92	2.23