



Camouflage by Integumentary Wetting in Bark Bugs

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more restrictive than those of the other (8). Here, too, the favored direction of junctional transmission [LRB(Glu)OH] was from the B cell to the partner.

A permeability asymmetry has two requisites. There must be a spatial asymmetry of channel structure, and this must give rise to an asymmetry of interaction between permeant and channel (21). The asymmetry condition here presumably is given by the pairing of unequal protochannels. We note in this connection that this condition is not met by merely pairing two different cell membranes; directional permselectivities were not apparent when we built heterologous junctions with mammalian cell membranes of equal homologous junctional permeability [tested on cell types with channels of permeability group II (8)]. As to the nature of the interaction, our earlier results with the homologous junctional Balb/c channel, probed with molecules of a wider range of sizes and charges (8), lead us to believe that it is either electrostatic, namely, an interaction with a fixed or induced charge guarding the channel, or a hydrogen bonding with the channel—and here one thinks first of the channel mouth as the locus of interaction.

An asymmetrically permeable cell-cell channel of the sort we have described has interesting physiological potential. If it occurs in heterologous junction of organs or tissues—and heterologous junctions are abundant—it could set up subtle internal boundaries where the traffic of certain molecules is effectively one-way. Selective boundaries of this sort could be important for partitioning tissues into cell domains of different physiology or different developmental fate. There is evidence that some domains of this kind are coupled by permeable junction (2, 4, 22). Little is known about the permeabilities of the channels at such boundaries, but it would be surprising if they were not somehow more limited than within the domains.

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10. With very small junctional potentials, conductance is symmetric in these rectifying junctions: E. J. Furshpan and D. D. Potter, *J. Physiol. (London)* **145**, 289 (1959); A. Auerbach and M. V. L. Bennett, *J. Gen. Physiol.* **53**, 211 (1969); see also T. G. Smith and B. T. Bauman, *Prog. Brain Res.* **31**, 313 (1969); J. Nicholls and D. Purves, *J. Physiol. (London)* **209**, 647 (1970); C. Slack and A. E. Warner, *ibid.* **248**, 97 (1975).
11. The Balb/c cells (from confluent stock) were seeded in plastic dishes together with the B cells or 18 to 24 hours after seeding the latter. Junctional permeability was tested on subconfluent cocultures 18 to 24 hours after the last seeding. One of the cell types (alternated) was labeled, before coculture, with 1- μ m latex particles, which the cells took up from the medium (Fig. 1). Besides, there was a natural marker; the Balb/c nucleus is granular. The growth medium was Eagle-Dulbecco's with 10 percent calf serum (Balb/c) or fetal calf serum (B). For coculture we used one serum or the other, with similar results.
12. The half-times of tracer fluorescence loss, determined by means of a photodiode system that images the cells through the microscope, were > 30 minutes (continuous illumination); junctional transit times were < 20 seconds; where junctional transit was scored negative, observation times were at least 10 minutes. The photodiode system was designed by R. Rick, B. Rose, and J. Gray. For a description, see J. L. Flagg-Newton and W. R. Loewenstein [*J. Membr. Biol.* **50**, 65 (1979)]. The fluorescences of the first-order neighbors (invariably greater than those of second-order ones) were averaged in the various trials. Deviations from the mean, in terms of our scale, were $\leq 1+$.
13. A junction here means two cells in contact as seen in phase contrast. Injected cells were multi-junctional, each trial giving information on several junctions. The acceptance criterion for a positive score was a minimum of two fluorescent first-order neighbors to make sure that the tracer had not passed between incompletely divided daughter cells. The soundness of this criterion was checked by injecting C-fluorescein together with the larger LRB(Leu)₂(Glu)₂OH (1158 daltons; Leu = leucine), which is mammalian-channel impermeant (8); there was no instance of transfer of the larger molecule through junctions scored positive for C-fluorescein transfer. For properties and purification of the tracers, see I. Simpson, B. Rose, and W. R. Loewenstein [*Science* **199**, 294 (1977)] and S. J. Socolar and W. R. Loewenstein [*Methods Membr. Biol.* **10**, 121 (1979)].
14. Electrical measurements were taken simultaneously with tracer injection on 22 cell pairs. Electrical coupling was symmetric within the resolution of the method even in conditions, as in Fig. 1, IIb, where LRB(Glu)OH failed to pass altogether from Balb/c to B.
15. The transfer asymmetry is apparent by both cumulative indices. In particular, the frequency distribution in Table 2 reveals that although not a single junction exhibited LRB(Glu)OH transfer from Balb/c to B in 27 of 48 trials, all trials scored > 48 percent permeable junctions in the opposite direction.
16. B. Rose, I. Simpson, W. R. Loewenstein, *Nature (London)* **267**, 625 (1977).
17. Of ten cell pairs thus examined, nine transmitted LRB(Glu)OH or FITC(Glu)OH from B to Balb/c with strong first-order neighbor fluorescence (+++); and of these same nine pairs, only one transmitted in the reverse direction and just detectably (+). In another control series, the Balb/c neighbor was simply impaled on a microelectrode (without injection) while the B cell was injected with LRB(Glu)OH; of the 11 pairs examined, all transmitted the tracer from B to Balb/c (+++).
18. The resting potentials of contiguous cells were not sensibly different. The permeability asymmetry was present when the tracers were iontophoresed with trains of (hyperpolarizing) pulses (2.5 to 10 nA, 10⁻² second, five per second) of durations three orders of magnitude shorter than the tracers' junctional transit times as well as with pulses of longer durations, and in conditions of voltage symmetry, when the tracers were pulsed simultaneously into two heterotypic cells (17).
19. Intracellular diffusion velocities were determined by imaging two regions of a given (large) cell, separated by a known distance, on an array of two photodiodes. The diffusion constants in a given cell type varied ≤ 8 percent and did not differ significantly in Balb/c and B cells, thus excluding the possibility of asymmetries arising from differences in tracer binding to cytoplasm or intracellular sequestering.
20. No double-impalement controls (17) were made in this series.
21. It follows from first principles (thermodynamics) that directional selectivity in passive diffusion can result only through an interaction of the permeant with the channel, or with another permeant species if the independence principle is violated. (An analysis of this point by J. N. Barrett is in preparation.)
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23. We thank I. Simpson for tracer synthesis, J. N. Barrett and S. J. Socolar for valuable discussion, J. Gray and C. Freitas for construction of apparatus. J.F.-N. held postdoctoral fellowship 5F32 GM05802 from the National Institutes of Health. The work was supported by research grant CA14464 from the National Cancer Institute.

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Camouflage by Integumentary Wetting in Bark Bugs

Abstract. Unlike most insect integuments, the body surfaces of certain bark-inhabiting bugs are wettable. A thin film of water reduces the reflectivity of the insect, resulting in a close match with the wettable bark upon which it rests. Wettability probably aids in concealing the insects from predators.

Insects that spend much of their lives exposed on tree trunks provide some of the best examples of camouflage in the animal kingdom. Pattern and resting behavior together serve to hide many nocturnal Lepidoptera from diurnal predators (1). The protective adaptations of

nonlepidopterous bark-resting insects are less well known. We now report a method of background matching in certain true bugs (Heteroptera) which compensates automatically for changes in background reflectance.

When wet with rain, the bark of trees

is noticeably darker than when dry. This change in reflectance (similar to the more familiar darkening of wet asphalt) increases the color saturation of reflected light reaching the observer. Most terrestrial insects, however, do not undergo this reflectance change, apparently because their integuments are not wettable. The unwettability of most insect integuments is caused by an epicuticular layer of wax or other hydrophobic molecules (2) that both protects them from desiccation and makes them less subject to the surface forces of aqueous media which they may contact.

We were surprised, therefore, to notice a change in the reflectance of certain bark-inhabiting Heteroptera after rain (3). The most dramatic cases were exhibited by *Dysodius lunatus* Fab. (Ara-didae), a common neotropical bark bug, and *Ceratozygum horridum* (Germar) (Pentatomidae), a neotropical stinkbug (Fig. 1). Experiments with light-colored

Table 1. Absolute percentage reflectance (R) of dry and wet bugs and bark (4) (mean \pm S.D.). The relative change in the average reflectance, $(R_{\text{dry}} - R_{\text{wet}})/R_{\text{dry}}$, is a more meaningful measure considering the logarithmic responses of visual systems.

State	Bark	Bugs	
	<i>Cecropia</i> sp.* (%)	<i>D. luna-tus</i> † (%)	<i>C. horri-dum</i> † (%)
	Absolute reflectance		
Dry	13.7 \pm 3.2	11.5 \pm 2.3	13.7 \pm 7.8
Wet	8.1 \pm 2.7	7.9 \pm 1.5	6.7 \pm 2.6
	Relative change in average reflectance		
	41	31	51

* $N = 20$. † $N = 10$.

living individuals touched to water demonstrated a sudden drop in relative reflectance of as much as 62 percent (Table 1) (4), as a film of water raced across the surface of the integument. Individuals contacting raindrops (5) or water streaming down tree trunks darken immediate-

ly. The change is fully reversible: upon drying, individuals of both species return to their lighter coloration.

No wettability differences were found between living and dead bugs, indicating that the property is one of the integument itself, not the living insect. It is not clear to what extent wettability is due to a hydrophilic surface, capillarity of ultrastructural sculpturing, setae and scales, or a combination of the two, but the effect is analogous to the converse situation in many aquatic insects, in which a thin film of air is held under water by the integument surface (6).

Dysodius and *Ceratozygum* differ from one another in the extent of the reflectance change. In *Dysodius*, the entire dorsal surface darkens upon wetting. The dorsal body surface of *Ceratozygum* is covered with minute white and ochre scales; when wet, these are optically "cleared" and the integument beneath shows through. Since the scales and the

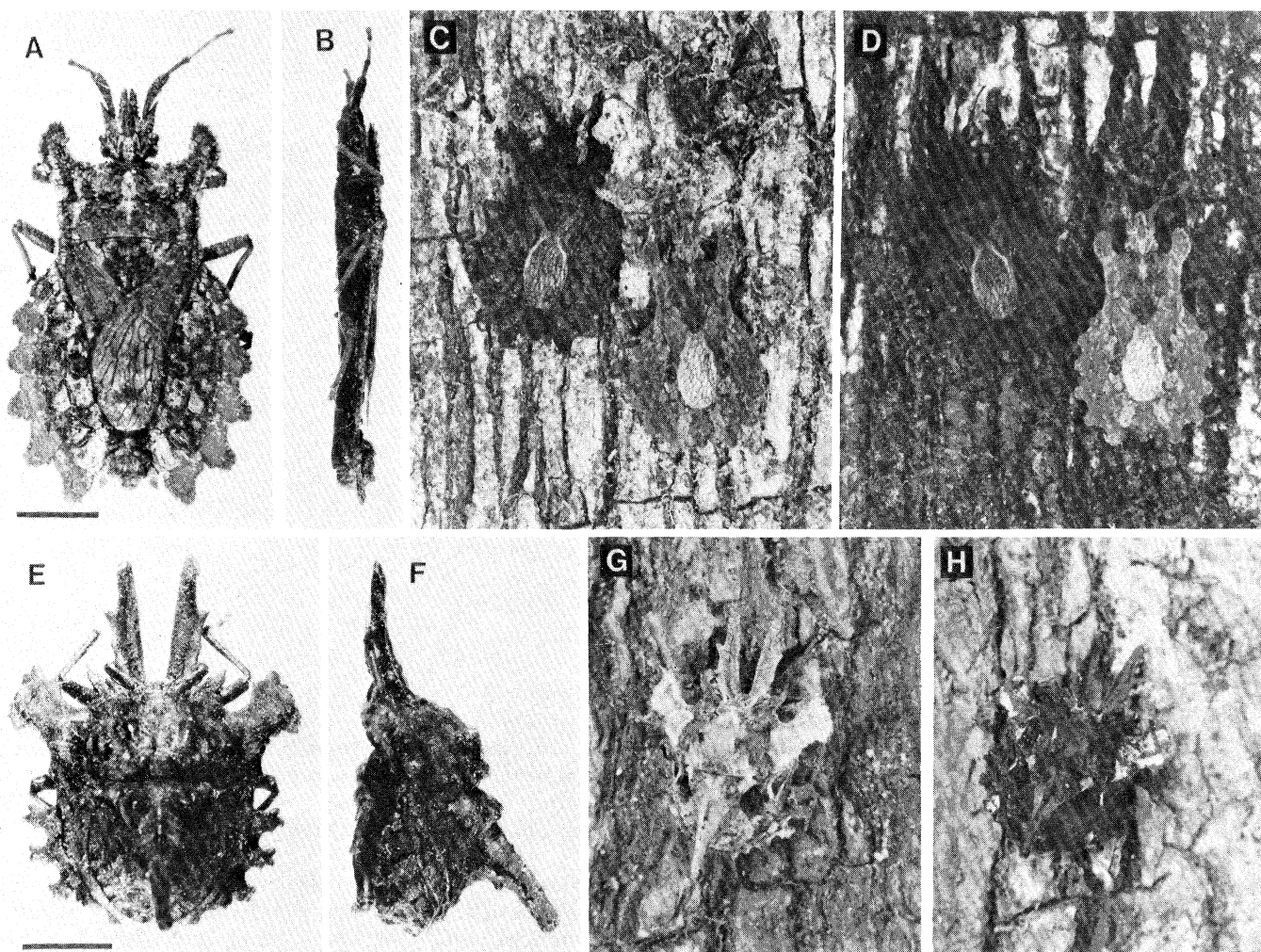


Fig. 1. (A and B) *Dysodius lunatus*, dorsal (A) and lateral (B) views (scale, 3 mm). (C and D) Pair of one wet and one dry *Dysodius* on dry (C) and wet (D) bark of *Cecropia* sp. The dry individual is inconspicuous on dry bark but easily discernible on wet; the wet bug is better camouflaged on wet bark than on dry. (E and F) *Ceratozygum horridum*, dorsal (E) and lateral (F) views (scale, 3 mm). (G and H) Dry (G) and wet (H) *Ceratozygum*, both on dry bark of *Cecropia*. There is a difference in pattern (especially on the pronotal lobes) as well as a change in reflectance.

integument are similar in color in some areas, but not in others, wetting changes not only the overall reflectance of the insect but its pattern as well (Fig. 1, G and H).

The ability to change reflectance in parallel with their substrate probably protects these bugs from visually oriented predators. Many bark-living Heteroptera, especially Aradidae, are slow-moving. When feeding, they cannot move to escape potential predators until their elongate mouthparts are extricated from the wood. Camouflage, aided by their coloration, often flattened shapes, and quiescent behavior, plus exocrine glands in some species, are their main lines of defense (7, 8). Wetting and darkening of tree trunks by rain would destroy the protective value of the insects' coloration. In these two species, effective camouflage is maintained in spite of such changes in background reflectance, simply by being wettable (9).

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 3. The observations were made on Barro Colorado Island, Panamá.
 4. Estimates of reflectance between 400 and 700 nm were made photographically by densitometric comparison of negatives with simultaneously photographed white-standards attenuated by stepped neutral-density filters. Details of this technique are given by R. E. Silberglied [*Functional Photogr.* 11 (No. 2), 20 (1976); *ibid.* 11 (No. 3), 30 (1976); K. Daumer, *Z. Vgl. Physiol.* 41, 49 (1958)].
 5. Unlike many of their near relatives, *Dysodius* and *Ceratozygum* have enlarged pronotal lobes (and, in *Ceratozygum*, elongate cephalic horns) that are usually the first parts of the body to enter water droplets.
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 7. Many bark-inhabiting Heteroptera (including some Aradidae) affix particles of bark or soil to the dorsal surfaces of their bodies, gaining both visual camouflage and the physical properties of their coverings, including wettability.
 8. R. L. Usinger and R. Matsuda, *Classification of the Aradidae (Hemiptera-Heteroptera)* [British Museum (Natural History), London, 1959], pp. 35-39. (Plate IV shows a dry *D. lunatus* on dry bark.)
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The Heart Is a Target Organ for Androgen

Abstract. Autoradiographic and biochemical analyses of the hearts of female rhesus monkeys and baboons indicate that atrial and ventricular myocardial cells contain androgen receptors. Although the specific effects of nuclear uptake and retention of androgen on the function of heart muscle cells are not known, the presence of this receptor suggests that sex steroid hormones may affect myocardial function directly and may explain some of the peculiar differences in heart disease between men and women.

Among the most puzzling features of coronary heart disease are the differences in morbidity and mortality from this disease in men and women. White men have more severe coronary artery atherosclerosis and more frequently experience myocardial infarction and sudden death than do white women. On the other hand, there is greater incidence of angina pectoris among women. These differences are not as great in nonwhite persons. There is little or no sex differential in other forms of arteriosclerotic heart disease.

In attempting to explain these relationships, emphasis has been placed on the possibility of a protective role of estrogen in reducing risk of arteriosclerotic disease, particularly through an effect on serum lipoproteins. However, the administration of estrogens to men who have experienced one myocardial infarct is accompanied by increased mortality; and the oral contraceptives, which have estrogenic activity, increase the risk of myocardial infarction in women, particularly in those who also smoke (1).

The observation that atrial, but not ventricular, myocardial cells possess specific estrogen receptors (2) indicates that the atrium may be affected directly

by circulating estrogenic hormones. We now have demonstrated androgen receptors in both atrial and ventricular myocardial cells of two species of nonhuman primates by both autoradiography and biochemical analyses, an observation suggesting that androgens also may affect cardiac function directly.

For these experiments we used six adult, normally cycling female rhesus monkeys (*Macaca mulata*) and baboons (*Papio cynocephalus*), three of each species. One animal of each set of three was a control. On day 1 of the experiment we removed both ovaries and the right adrenal gland from each animal. On day 3 we removed the left adrenal gland. At this second operation, the test animals received 100 mg of hydrocortisone. On day 4, we injected intravenously into the test animals 1 μ g of 5 α -dihydro[1, 2, 4, 5, 6, 7-³H]testosterone (³H]DHT) (101 Ci/mole) per kilogram of body weight. We injected the two control animals with the labeled material together with 100 μ g of unlabeled hormone per kilogram of body weight. One hour later, we exsanguinated each animal rapidly and perfused the vascular system with chilled Ringer solution. Tissue samples were mounted on tissue holders, frozen in liquefied propane, and stored in liquid ni-

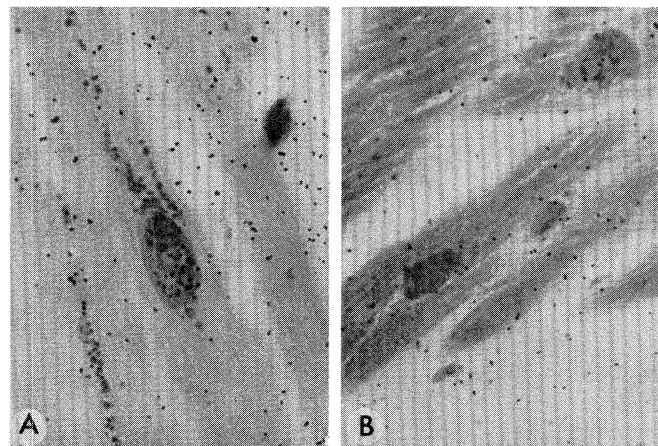


Fig. 1. Autoradiographs of baboon heart muscle. (A) Section from animal injected with [³H]DHT shows scattered silver grains due to free or bound steroid, with a marked concentration of grains over a nucleus of a myocardial fiber. Lighter gray granules adjacent to the nucleus are parts of sarcoplasm, not silver grains. (B) Section from a control animal injected with [³H]DHT and unlabeled DHT shows no nuclear localization of silver grains, and thus demonstrates saturability of binding by competition.