Table 1. Phenotypes and genotypes of donor and host animals.

Donors (all phenotypes white)		Hosts		
Strains	Genotype	Strains	Genotype	Phenotype
Lewis	$a/a;c^a/c^a$	Lewis × DA	$A^w/a;C/c^a$	Gray-bellied agouti
Lewis	$a/a;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black
(Lewis × DA)F_2	$A^w/A^w;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black
A (mouse)	$a/a;c^a/c^a$	Lewis × DA	$A^w/a;C/c^a$	Gray-bellied agouti
BALB/c (mouse)	$A/A;c^a/c^a$	Lewis × BN	$a/a;C/c^a$	Black

days old, to recipients less than 12 hours of age after birth (*3*). All grafts, which were about 0.7 to 0.8 cm in diameter, originated from dorsal regions and were placed in the mid-dorsal region of the host. The grafts were examined daily under a dissecting microscope. In all instances pigmented graft hairs, as well as host hairs, were plucked and examined microscopically (*4*).

In the intraspecific rat combinations tested, graft hairs began to emerge on the 12th day after surgery. Usually they were devoid of pigment. However, within a few days many pigmented hairs appeared within the graft. Most of them were located immediately within the graft border, but some were found well within its perimeter, intermingled with the characteristic nonpigmented donor hairs. These secondarily pigmented hairs increased in number so rapidly that by 25 days the pigmentation of the fur on the graft was sometimes discernible to the naked eye. Indeed, some grafts ultimately acquired as many pigmented as nonpigmented hairs.

In all instances the color of graft hairs populated by host melanocytes was determined by the agouti-locus genotype of the follicles. Thus, in six Lewis grafts to Lewis × BN (non-agouti) recipients, black hairs, or black-white mosaic hairs, emerged among the indigenous, nonpigmented hairs within the graft border. A similar situation occurred in eight Lewis grafts to Lewis × DA (agouti) animals. Eight Lewis × BN (non-agouti) hybrids tolerant of albino but agouti (A^w/A^w; c^a/c^a) grafts displayed agouti hairs among the white hairs. A number of completely black guard hairs were found as well, but these also occur normally in agouti animals.

As anticipated, and unlike rat skin homografts, mouse skin heterografts did not live very long on the rat hosts. Of 38 heterografts only 10 exhibited some evidence of epithelial survival at 20 days and in no instance did a mouse graft survive longer than 25 days on a rat. However, inasmuch as pigmented graft hairs were first observed as early as the 12th day after grafting, the short life-span of these heterografts did not preclude appraisal of the fate of rat melanocytes in mouse follicles.

The color of the pigmented hairs that originated within these heterografts was again determined by their agouti-locus genotype. Thus when strain A (albino but non-agouti) mouse skin was transplanted to Lewis × DA (agouti) rat hybrids, four animals displayed some black or gray hairs within the border of the graft. The greatest number of these hairs in any single recipient was about 10 to 12 and, in most instances, they were predominantly mosaic in nature, containing a reduced number of black granules. In all eight cases in which pigmented hairs were found within the border of albino but agouti mouse grafts (furnished by BALB/c animals) on non-agouti, Lewis × BN hosts, they were typical agouti in pattern, exhibiting black tips with yellow bands. Some of these hairs originated from grafts which were rejected while they were in the yellow phase but others survived long enough to revert back to eumelanin production. Many of these pigmented hairs were deficient in pigment granules, containing either a reduced number of granules in each hair septule, or, completely lacking granules over a portion of the shaft. This deficiency of pigment, like that noted in other combinations, was undoubtedly the result of the incorporation of only a few melanoblasts in the hair follicle. A few black hairs were also observed originating from these grafts but, as already stated, they are also found naturally in agouti animals. The number of graft hairs pigmented by host cells varied from two or three to more than 30.

These findings indicate that the primary action of genes at the agouti locus of the rat is the same as in the mouse—that is, in the hair bulb and not in the melanocyte. Furthermore, the agouti locus in these two species is apparently homologous inasmuch as rat melanocytes can respond to the agouti-locus genotype of mouse hair follicles. This situation is certainly unique and, as far as is known, represents the first example in vivo where the behavior of a cell of one species is completely determined by the genotype of an alien host species.

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Progesterone: Biosynthesis from Pregnenolone in Holarrhena floribunda

Abstract. *After administration of pregnenolone-4-C^{14} to* Holarrhena floribunda *leaves, radioactive progesterone was isolated and purified to constant specific activity by chromatography, conversion to a derivative, and recrystallization. The result suggests that the biogenetic sequence leading to progesterone is the same in plants as in animals.*

The discovery of the animal hormone progesterone in the African plant *Holarrhena floribunda* (*1*) has led us to study the biosynthesis of this compound. In animals, progesterone is formed directly from pregnenolone (*2*). The latter has thus far been found in only two plants, *Xysmalobium undulatum* (*3*) and *Trachycalymna fimbriatum* (*4*). However, pregnenolone-21-C^{14}-glucoside was converted to digitoxigenin and other cardenolides by *Digitalis lanata* leaves (*5*), and Tschesche and Snatzke (*3*) have suggested that pregnenolone may serve as a precursor of the steroidal estrogens found in plants (6), as it does in animals (*2*). We have now isolated

radioactive progesterone from *Holarrhena floribunda* leaves treated with pregnenolone-4-C^{14}.

Pregnenolone-4-C^{14} (New England Nuclear, 46 μc/μmole) was applied in acetone solution (20 μl, 280,000 count/min) to several leaves of a *Holarrhena floribunda* plant (*7*) growing in soil. The leaves were then sprayed with a solution of silicone oil in petroleum ether to promote absorption of the pregnenolone (*8*). A total of ten such treatments were given, three per week. Three days after the last treatment, the leaves were removed and lyophilized. The dried leaves (0.5 g) were homogenized with water at *p*H 9 to 10, the solution was separated by centrifugation, and the residue was reextracted with water. The aqueous solutions were combined and extracted with dichloromethane. The leaf residue was extracted with boiling acetone, and the two organic extracts were combined and freed of alkaloids by extraction with 0.5*N* HCl (*9*).

The neutral fraction (1,490,000 count/min), weighing 37 mg, was examined by thin-layer chromatography on a silica-gel G plate (50 × 200 mm), 0.3 mm thick, which was developed with a mixture of dichloromethane and methanol (97 : 3). A scan of the radioactivity showed four peaks, the major one being associated with unchanged pregnenolone (R_F, 0.32). The other three were located at R_F of 0.00, 0.14, and 0.56, the last coinciding with progesterone. Since neither pregnenolone nor progesterone was present in the extract in quantity sufficient for detection by spraying with 50-percent sulfuric acid, cochromatographed standards were used to locate their positions on the chromatogram.

By preparative thin-layer chromatography of one-fourth of the neutral fraction in the same system, the radioactive zone corresponding to progesterone was isolated (8800 count/min). The material in this zone was subjected to thin-layer chromatography on a plate, as described, with a mixture of hexane and ether (3 : 7). Two radioactive peaks were located by a scanning procedure, the major peak having the same mobility as progesterone (R_F, 0.25) and the other having an R_F of 0.14. The zone corresponding to progesterone was removed and eluted (4800 count/min). Thin-layer chromatography of a portion of this material on a silica-gel G plate, developed continuously (*10*) with a mixture of hexane and ether (4 : 1) for 8 hours, showed only a single radioactive peak, coinciding with progesterone.

The remainder of the progesterone zone was treated with 2,4-dinitrophenylhydrazine in methanol. The product was chromatographed on a plate, as above, with a mixture of carbon tetrachloride and dichloromethane (1 : 9) being used for development. No radioactivity was associated with the zone of the chromatogram corresponding to progesterone (R_F, 0.08), and the only peak revealed by scanning coincided with a cochromatographed sample of progesterone bis(2,4-dinitrophenylhydrazone) (R_F, 0.48) prepared from authentic progesterone (*11*). This zone was removed and eluted (3500 count/min). A portion of this was diluted with pure progesterone bis(2,4-dinitrophenylhydrazone) and recrystallized from a chloroform-ethanol mixture. The crystals, having a specific activity of 34.9 ± 1.7 count min^{-1} $\mu mole^{-1}$ (*12*), were recrystallized from the same solvent with no change in specific activity (35.2 ± 1.7 count min^{-1} $\mu mole^{-1}$). The specific activity also remained constant after recrystallization from a chloroform-benzene mixture (34.8 ± 1.7 count min^{-1} $\mu mole^{-1}$). Finally, hydrolysis to progesterone with chromous chloride and HCl (*13*), followed by recrystallization from hexane, did not alter the specific activity (34.3 ± 1.7 count min^{-1} $\mu mole^{-1}$).

The incorporation of pregnenolone into progesterone was low (<1 percent), and a further experiment demonstrated that the latter is rapidly metabolized by the plant. Progesterone-4-C^{14} was administered to a second *H. floribunda* plant, exactly as in the pregnenolone experiment above, and the leaves were worked up in the same manner. Only 3 percent of unchanged progesterone was recovered. Thus, the conversion of pregnenolone to progesterone must have been much higher than the incorporation rate indicates.

This experiment, together with previous work on the biosynthesis of steroids from acetate, mevalonic acid, and squalene (*8*, *14*), indicates that the pathway of biosynthesis of progesterone is probably the same in plants as in animals. However, its physiological function, if any, in plants remains to be determined.

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Aggressive Mimicry in Photuris: Firefly Femmes Fatales

Abstract. *Firefly females of the genus* Photuris, *long known to be carnivorous, attract and devour males of the genus* Photinus *by mimicking the flash-responses of* Photinus *females. Although suspected, this behavior had not been observed previously.*

While observing firefly behavior, several naturalists have noted that females of the genus *Photuris* are carnivorous. Many, including myself, have discovered this by trying to keep groups of fireflies alive overnight in the same container. In the morning one usually finds one *Photuris* female and bits and pieces of all the rest. Barber (1) observed *Photuris* females in spider webs eating glowing fireflies that had been captured and wrapped by spiders. He also observed courting males of the genus *Photinus* receiving flashed responses from perched *Photuris* females. He asked: "Does she lure him to serve as her repast?" During the past three summers while working in the field on flash-communication in the firefly genus *Photinus*, I have made several observations