twitch suggests renewed synthesis and release of catecholamines. This decline in contracture and the shortening of twitch was not observed when the site of drug action was blocked by propranalol.

Since a primary role has been attributed to the sarcoplasmic reticulum in the relaxation process (*6*), it becomes necessary to ask whether the relaxation effect of catecholamines is mediated through the sarcoplasmic reticulum or through some other mechanism. The ease of eliciting contractures in the frog heart, a tissue lacking in sarcoplasmic reticulum (*7*, *8*) could be suggestive. However, it must be remembered that epinephrine also relaxes the contracture induced by KCl in the frog ventricles (*3*). Other biochemical evidence indicates that both reserpine at $4 \times 10^{-4}M$ and propranalol at $4 \times 10^{-3}M$ inhibit the calcium-pumping activity of the isolated vesicles [skeletal muscle (*9*) and cardiac muscle (*10*, *11*)] which was not reversed by catecholamines (*11*). However, the concentrations of these agents used here were of the order of 1000 times less, and propranalol at $10^{-6}M$ had no negative inotropic effect. Furthermore there is no biochemical evidence yet available to suggest an enhancement of calcium-pumping activity of the isolated vesicles in the presence of catecholamines (*10*). In fact there is some evidence that catecholamines may inhibit calicum-pumping of the sarcoplasmic reticular fraction (*12*). Electronmicroscopic examination of the preparations showed no gross differences in the sarcoplasmic reticulum or mitochondrial appearance of hearts treated with reserpine or propranalol when compared to the nontreated myocardium.

The role of catecholamines in the myocardial contractility, regardless of their mechanisms of action, may be intimately related to the process of relaxation. The shortening of the duration of contraction produced by epinephrine, in spite of an increase in the duration of the action potential (*3*, *13*), may well reflect the beat-to-beat relaxation-stimulating action of catecholamines that is analogous to its effect to relax contracture. In the mammalian heart, where the sequestering of calcium must be efficient, the calcium-pump may be doubly protected by: (i) The increased total membrane surface, for example, well-developed sarcoplasmic reticulum and (ii) an abundance of catecholamines through rich sympathetic innervation. It is this very efficient calcium-pump that is able to handle the extra load of calcium during a challenge with the solutions that produce contractures. The diminished sequestering surface (for example, with frog ventricle) or the removal of the "pump-stimulant" (catecholamines) is sometimes sufficient to slow the transport system just enough to throw the muscle into contracture (a state of calcium overload) upon exposure to KCl solutions.

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References and Notes

1. M. O. Schilling, *Rev. Sci. Instrum.* **31**, 1215 (1960).
2. J. Koch-Weser, *J. Pharmacol. Exp. Ther.* **150**, 184 (1965).
3. F. Kavaler and M. Morad, *Circ. Res.* **18**, 492 (1966).
4. A. J. Graham and J. F. Lamb, *J. Physiol.* **197**, 479 (1968).
5. G. Haeusler, H. Thoenen, W. Haffely, A. Huerlimann, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **261**, 389 (1968).
6. W. Hasselbach, *Progr. Biophys. Mol. Biol.* **14**, 169 (1964).
7. R. Niedergerke, *J. Physiol.* **167**, 515 (1963).
8. N. A. Staley and E. S. Benson, *J. Cell Biol.* **38**, 99 (1968).
9. H. Balzer, M. Makinose, W. Hasselbach, *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **260**, 444 (1968).
10. M. L. Hess and F. N. Briggs, *Nature* **220**, 79 (1968).
11. B. Scales and D. A. D. McIntosh, *J. Pharmacol. Exp. Ther.* **160**, 261 (1968).
12. D. H. Yu and S. Triester, *Fed. Proc.* **28**, 542 (1969).
13. M. Morad, *Pflügers Arch. Gesamte Physiol. Menschen Tiere* **289**, 19 (1966).
14. I thank Drs. A. J. Brady and G. Langer for discussions and advice and R. Orkand for reading the manuscript. Supported by American Heart grant 4-444968-58138.

21 April 1969; revised 22 July 1969

Flower of Tetraplasandra gymnocarpa Hypogyny with Epigynous Ancestry

Abstract. *Comparative herbarium studies, floral anatomy, and distributional data show that* Tetraplasandra gymnocarpa, *an araliad with hypogynous flowers, evolved in Hawaii from ancestors with epigynous flowers. Suggested causes for this reversal of a well-known evolutionary trend are (i) isolation of the ancestors from flower-eating predators and (ii) selection for increased outcrossing.*

A flower is hypogynous if its sepals, petals, and stamens radiate from below the locules, or seed cavities. The ovary, which encloses the locules, is said to be superior when the flower is hypogynous. A flower is epigynous if its sepals, petals, and stamens diverge above the locules and therefore appear affixed to the summit of the (inferior) ovary. The change from hypogyny to epigyny, having occurred independently in many families, is one of the firmly established evolutionary tendencies in flowering plants. We report an example of the reverse change, presumably the first example of secondary hypogyny to be recognized (*1*).

The reversal has taken place in the Araliaceae, a predominantly tropical family whose most familiar member (to residents of northern temperate regions) is English ivy. All but a few of the hundreds of species of Araliaceae have markedly inferior ovaries, and completely superior ovaries occur only in *Tetraplasandra*, a genus of trees of Pacific islands.

The geographic distribution of *Tetraplasandra* and comparative anatomy of the flowers (*2*) provide the evidence for the secondary origin of the superior ovary. Twenty species are found only on the Hawaiian islands (*3*); the others—probably only two in number (*4*)—are confined to islands of the western Pacific: New Guinea, Celebes, Palawan in the Philippines, and San Cristóbal in the Solomons (*5*). Because of the relative geologic youth of the Hawaiian chain, one would expect the western species to be older, and floral structure confirms this expectation. Flowers of *T. paucidens* Miq. from New Guinea have the following structural features (Fig. 1) that most plant systematists would accept as primitive: (i) stamens and carpels are numerous (*6*); (ii) carpel margins are unsealed above the locules, and their apical (stigmatic) portions unite around an open stylar canal; (iii) dorsal bundles of the carpels are separate, at least in part, from bundles supplying the sepals, petals, and stamens; (iv) some of the ventral bundles are in pairs. Hawaiian tetraplasandras are thought to have originated from one ancestral colonizing species (*7*), and our studies indicate that *T. hawaiensis* A. Gray is the modern species closest to the ancestor. A species of broad ecological amplitude (*8*), *T. hawaiensis* is found on four of the Hawaiian islands; moreover, it has many resemblances to tetraplasandras of the western Pacific, including the floral characters listed above (*9*).

Flowers of *T. hawaiensis*, like those of the western Pacific species, are epigynous. There are two species of *Tetraplasandra* endemic to Oahu, however, in which the ovaries are superior at the time of flowering and remain

Table 1. Range in numbers of floral parts in selected species of *Tetraplasandra*. Data combine original observations with observations of other authors.

Species	Petals	Stamens	Carpels
Western Pacific species	7–9	20–40+	7–13
T. hawaiensis	6–8	20–30	6–13
*T. gymnocarpa**	5–9	5–9	2–5
*T. kavaiensis**	6–9	6–9	2–5

* Stamens isomerous with petals.

superior as fruits develop. We have examined floral material from one of these, *T. gymnocarpa* (Hillebr.) Sherff, a small rainforest tree. Floral appendages are relatively few in *T. gymnocarpa* (Table 1), and internal floral characters (Fig. 1) are more advanced than those of *T. hawaiensis*. For instance, the ventral vascular supply consists of one bundle for each carpel; there are no pairs. Also, the carpel margins are not open above the locules; instead, the apical portions of the carpels are so intimately united that the pollen-transmitting tract can scarcely be identified in some cross sections, and the stigmas are mere depressions in the domelike summit of the ovary.

Ironically, the secondary origin of the superior ovary has heretofore passed unnoticed because most taxonomic treatments have overemphasized the superior ovary, with *T. gymnocarpa* being assigned to a separate genus (*Pterotropia* or *Dipanax*). The error of these treatments is demonstrated by *T. kavaiensis* (H. Mann) Sherff, a species intermediate between *T. gymnocarpa* and *T. hawaiensis*. The floral structure of *T. kavaiensis* is like that of *T. gymnocarpa*, except that the ovary is only partially superior. In stature and in the hairiness of its leaves, *T. kavaiensis* resembles *T. hawaiensis*. Furthermore, the three species have in common a loosely branched inflorescence in which only the outermost flowers tend to radiate in the umbrella-like form typical of Araliaceae. In this respect they resemble tetraplasandras of the western Pacific more than they resemble their nearest Hawaiian relatives.

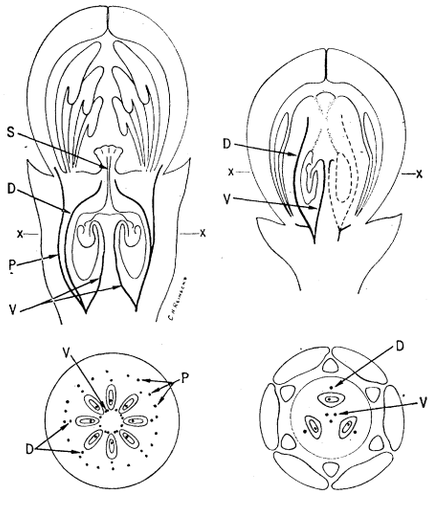

Fig. 1. Flower of *Tetraplasandra paucidens* (left) and *T. gymnocarpa* (right); diagrams show cross section and median longitudinal section. Parts of the vascular system are omitted to emphasize features mentioned in text; for instance, only two of the many peripheral bundles (*P*) supplying sepals, petals, and stamens are indicated in the longitudinal section of *T. paucidens*. Actual length of flower about 5 to 10 mm. *D*, Dorsal bundles; *S*, stylar canal; *V*, ventral bundles; *xx*, plane of cross section.

The genetic readjustments required for reversal from hypogyny to epigyny (*10*) are obviously not very great in *Tetraplasandra*. This may also be true of Araliaceae in general, for partially superior ovaries occur in a few of the other genera (*11*)—and in at least one genus of the related family Umbelliferae (*12*). These taxa are not closely related to each other, and some of them occupy a relatively advanced position in the alliance; therefore it is likely that in each case the partially superior condition has evolved independently from epigynous ancestry.

One factor that can be invoked to account for the complete resumption of hypogyny in *T. gymnocarpa* is the lesser amount of pest pressure encountered by a species that colonizes an island far from its original range. Epigyny is believed to have evolved—at least in most cases—in response to natural selection by insects and other animals that eat floral parts (*13*). That is, seeds developing in an inferior ovary are thought to be protected from these predators when superior parts are eaten. If this is true, the separation of the plants from the predators by dispersal to a new island habitat would remove the selective pressure against mutants with superior ovaries.

Selection for increased outcrossing —an important evolutionary process on oceanic islands (*14*)—may also have played a part in the secondary derivation of the superior ovary. Flowers of *T. hawaiensis* are well constructed for self-pollination. Pollen is released by the anthers before the flowers are completely open, and the position of the anthers above the open stylar canal would seem to insure that all ovules are supplied with pollen grains. In buds of *T. gymnocarpa*, however, the summit of the ovary is separated from the anthers, and the stigmas, where pollen would be expected to enter, are pressed against the enclosing petals. Although this arrangement seems admirably adapted for preventing self-pollination, we are obliged to add a cautionary note because of an apparent contradiction in our observations. To judge from the herbarium specimens we have examined, the petals of *T. gymnocarpa* do not separate, but remain united while the pollen is shed, eventually falling away as a sort of cap, the dehisced stamens adhering to the inside of the cap when it falls. We wonder therefore how much cross-pollination actually occurs in *T. gymnocarpa*. Clearly, this question cannot be answered by herbarium studies; we must leave it to those who have access to living plants.

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References and Notes

1. S. G. Tamamshyan's "Secondary hypogyny of the flower of Umbelliferae . . ." [*Dokl. Akad. Nauk SSSR* **61**, 537 (1948), in Russian] has little or no bearing on our findings, even though it deals with close relatives of the Araliaceae. It is based on a concept of the inferior ovary that was once current in the Soviet Union but has since been repudiated: see, for instance, N. V. Pervukhina, *Akad. Nauk SSSR, Tr. Bot. Inst.*, ser. 7, **5**, 31 (1962); A. L. Takhtadzhyan, *Osnovy Evolyutsionnoi Morfologii Pokrytosemennykh* (Akad. Nauk SSSR, Moskva-Leningrad, 1964), pp. 102–107.
2. The observations on *Tetraplasandra* emerged from a family-wide investigation of primitive versus derived floral structure in Araliaceae. Our methods are conventional—dissection, serial microtome sections, cleared whole mounts. Lacking fluid-preserved specimens of *Tetraplasandra*, we used herbarium flowers; vouchers will be cited in our final report.
3. E. E. Sherff, *Fieldiana Bot.* **29**, 49 (1955), recognized 19 species; another was added by O. Degener and I. Degener, in O. Degener, *Flora Hawaiiensis*, Book 6, Fam. 281 (Honolulu, 1962). Sherff's treatment probably overemphasizes minor differences, but it is convenient to follow, because most herbarium collections bear his annotations.
4. W. R. Philipson, *Bull. Brit. Mus.* (*Natur. Hist.*) *Bot.* **1**, 3 (1951).
5. Also on the tiny island Dwars in the Sunda Strait, according to F. A. W. Miquel, *Ann. Mus. Bot. Lugduno-Batavi* **1**, 4 (1863).
6. A. Cronquist, in his *Evolution and Classification of Flowering Plants* (Houghton-Mifflin, Boston, 1968), has challenged the view, long held by specialists in Araliaceae, that flowers with numerous parts are primitive. Our studies support the older opinion.
7. F. R. Fosberg, in E. C. Zimmerman, *Insects of Hawaii* (Univ. of Hawaii Press, Honolulu, 1948), vol. 1, p. 107.
8. J. F. Rock, *The Indigenous Trees of the*

Hawaiian Islands (Published under Patronage, Honolulu, 1913), pp. 336–365.
9. S. Carlquist [*Brittonia* **18**, 317 (1966)] uses a similar geographic comparison to show that species of *Tetraplasandra* with large flowers and fruits are derived from species with small flowers and fruits.
10. G. L. Stebbins, Jr. [*Evol. Biol.* **1**, 137 (1967)] discusses the probable genetic basis for the near irreversibility of hypogyny-epigyny and similar evolutionary trends.
11. Examples are *Schefflera* and *Hedera*. See H. Harms, in A. Engler and K. Prantl, *Die Natürlichen Pflanzenfamilien*, Teil III, Abt. 8 (1894), p. 8; N. V. Pervukhina, *Bot. Zh.* **38**, 188 (1953).
12. *Heracleum*. See, for example, E. Liehr, *Mitt. Inst. Allgem. Bot. Hamburg* **6**, 365 (1927); V. G. Aleksandrov and N. V. Pervukhina, *Akad. Nauk SSSR, Tr. Bot. Inst.*, ser. 7, **3**, 5 (1952).
13. V. Grant, *Evolution* **4**, 179 (1950).
14. S. Carlquist, *Quart. Rev. Biol.* **41**, 263 (1966); *Evolution* **20**, 433 (1966).
15. Supported in part by grants from the Smithsonian Research Foundation (to R.H.E.) and the NSF (to C.C.T.). Drawings by C. Reinecke. We thank J. Morgan and C. Warner for microtechnical assistance, H. Robinson and W. R. Philipson for reading the typescript.

29 May 1969

Cardiac Sympathetic Nerve Activity: Changes Induced by Ouabain and Propranolol

Abstract. *A study of the effects of ouabain and propranolol on the spontaneous activity in the preganglionic sympathetic nerves to the cat heart showed that ouabain can produce both an inhibition and a stimulation of the spontaneous activity in sympathetic nerves. The inhibition appears to be reflex in nature and is not present when the buffer nerves are sectioned. The stimulation is correlated with the development of cardiac arrhythmias and is antagonized by propranolol.*

The role of the sympathetic nervous system in the cardiac actions of digitalis drugs has been studied by many investigators, but unanimity of opinion on it has not been forthcoming; indeed, controversy seems to describe best the present state of affairs (*1*). Thus, according to some, the sympathetic nervous system is activated by these drugs and the effects of this activation contribute to the contractile (*2*) and arrhythmogenic responses (*3*). According to others, the sympathetic nervous system is inhibited by the cardiac glycosides and the effects of this inhibition modify the heart rate (*4*), refractory period (*5*), and contractile responses (*6*); two of these authors (*6, 7*) reported that digitalis inhibits efferent discharges in the pre- and postganglionic stellate nerve fibers. Finally, some investigators maintain that these substances are not dependent on the sympathetic nervous system for their actions (*8*).

I have recently obtained direct evidence that ouabain can both inhibit and stimulate the adrenergic nervous system; the response is dependent on the functional state of the cardiovascular reflexes, and the stimulant effect is antagonized by propranolol. This report presents evidence for these effects of ouabain and propranolol on the activity in the preganglionic sympathetic nerves to the heart.

Experiments were carried out in cats decerebrated during ether anesthesia by a stereotaxically placed electrolytic lesion that transected the brain stem in the midcollicular plane. Denervation of the reflexogenic areas was performed in some animals by bilateral section of the carotid sinus and vagus nerves. All animals were ventilated mechanically with 97 percent oxygen and 3 percent carbon dioxide, and their rectal temperatures were maintained between 37° and 38.5°C with an infrared lamp. Femoral blood pressure and lead I or II of the electrocardiogram (ECG) were recorded continuously. Spontaneously occurring activity in the preganglionic nerves to the right stellate ganglion was recorded with bipolar platinum electrodes. The nerves from the ganglion to the heart were not cut; that these nerves innervated the myocardium was evidenced by the fact that their stimulation consistently increased heart rate. Ouabain was administered in single doses of 20, 50, or 100 μg per kilogram of body weight by injection through a cannula in the femoral vein.

The normal activity in these nerves can best be described as bursting and rhythmical. As shown in the A records (controls) of Fig. 1, the bursts of activity occur with a constant interval and frequency in each experiment and in synchrony with inspiration. The effects of ouabain on this activity in control animals (carotid sinus and vagus nerves intact) depend upon the time after injection and the size of the dose. Inhibition of the spontaneous sympathetic activity is the initial effect. It is produced by doses as low as 20 μg/kg and often is the only effect produced by these low doses. After doses in the range of 40 to 100 μg/kg, the initial inhibitory effect is greater, but as time after the injection increases, the spontaneous neural activity returns and increases far above the predrug control. In the inhibitory phase the sympathetic nerve activity is reduced in amplitude or abolished (Fig. 1, control B). The inhibition begins within 1 to 5 minutes, is maximum from 4 to 9 minutes after administration of the drug, and lasts 5 to 20 minutes, depending on the dose. It is immediately followed by a stimulatory phase (Fig. 1, control C) in which the bursts of activity reappear, increase in intensity and duration, and become arrhythmic. In addition, neural activity between the bursts begins and becomes increasingly intense. In a final stage, the burst and the interburst activities merge into a continuous stream of high-intensity neural activity; ventricular arrhythmias occur and the animal dies. After doses of 100 μg/kg the inhibitory phase is transient, or sometimes absent, and excitation is the predominant effect. In the 32 experiments performed, a biphasic response was observed in 23, inhibition only in four (in which only small doses were given), and stimulation only in five (in which only large doses were administered).

The changes in neural activity induced by ouabain do not seem to be related to changes in blood pressure. That is, the entire pattern may be observed in animals in which there is no appreciable change in blood pressure; in animals in which changes do occur, there is no consistent correlation between changes in blood pressure and changes in neural activity. Cutting the sympathetic nerve distal to the electrode does not change the neural activity or the effects of ouabain on this activity.

Denervation of the reflexogenic areas in the carotid sinuses and aortic arch prevents the inhibitory effect of ouabain on this neural activity (Fig. 1, denervated). The administration of 20 μg/kg of ouabain has no effect on the ongoing sympathetic discharge. Larger doses produce an increase in neural activity which is greater and occurs sooner after injection of the drug than doses of the same size produce in control animals; a dose of at least 50 μg/