

INCOMPATIBILITY SYSTEM IN *THEOBROMA CACAO*

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LETTERS TO THE EDITORS

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INCOMPATIBILITY SYSTEM IN *THEOBROMA CACAO**

The genetics of self-incompatibility in *Theobroma cacao* is considered unique among the identified systems in the flowering plants. Knight and Rogers (1955) reported that it has a sporophytic system controlled by one series of *S* alleles which show dominant or "equal" (independent) action in both male and female organs. However, pollen-tube studies showed that there is no inhibition of the pollen-tube growth in incompatible crosses and that the contents of the pollen-tube are liberated in the ovules, the ensuing failure to set fruit being due to a subsequent incompatibility. Further cytological studies by Cope (1958), revealed that in incompatible crosses, non-fusion of gametes may occur in 25, 50, or 100 per cent of the ovules. He proposed that the gametes that do not fuse in the embryo-sac are those carrying the same dominant allele. This gives the impression of a gametophytic control of incompatibility.

To explain the unusual situation in *T. cacao*, Cope postulated the existence of two independent loci: one (*P*, *p*), which controls the production of an incompatibility precursor, shows simple dominance and recessivity and acts before meiosis; the other (*S*₁, . . . *S*_n) imparts specificity to the precursor, shows allelomorphism and acts after meiosis. He suggested that the self-compatibility of the Central American and Trinidad populations of *T. cacao* is due to one or both loci being inactive (*pS*, *PS*_f or *pS*_f where *f* indicates the presence of an amorph). A cross between representatives of the above mentioned populations (believed to be *pS* and *PS*_f) gave all self-incompatible trees.

The experimental results of Knight and Rogers and Cope, leave no doubt about their conclusion regarding the physiological nature of the incompatibility system in this species. However, the genetic interpretation of these processes is unnecessarily complicated. A precocious (premeiotic) action of the specific (*S*) pollen growth substance producing unit of the *S* gene complex, when interaction between the two *S* alleles could occur, has been postulated in the hybrid between the gametophytically controlled self-incompatible *Oenothera pallida* (♂) and self-compatible *O. trichocalyx* (♀) (Crowe, 1955; Pandey, 1960). In this hybrid the *S* allelic specificity is superimposed upon specific pollen growth substances post-meiotically.

The sequence of events, regarding *S* gene action, in *T. cacao* may be assumed to be as follows (figure 1): (1) premeiotic production of specific growth substances leading to *S* allele interaction, (2) post-meiotic production of specific incompatibility precursors, (3) conversion of specific precursors into specific incompatibility substances in pollen-tubes in the male

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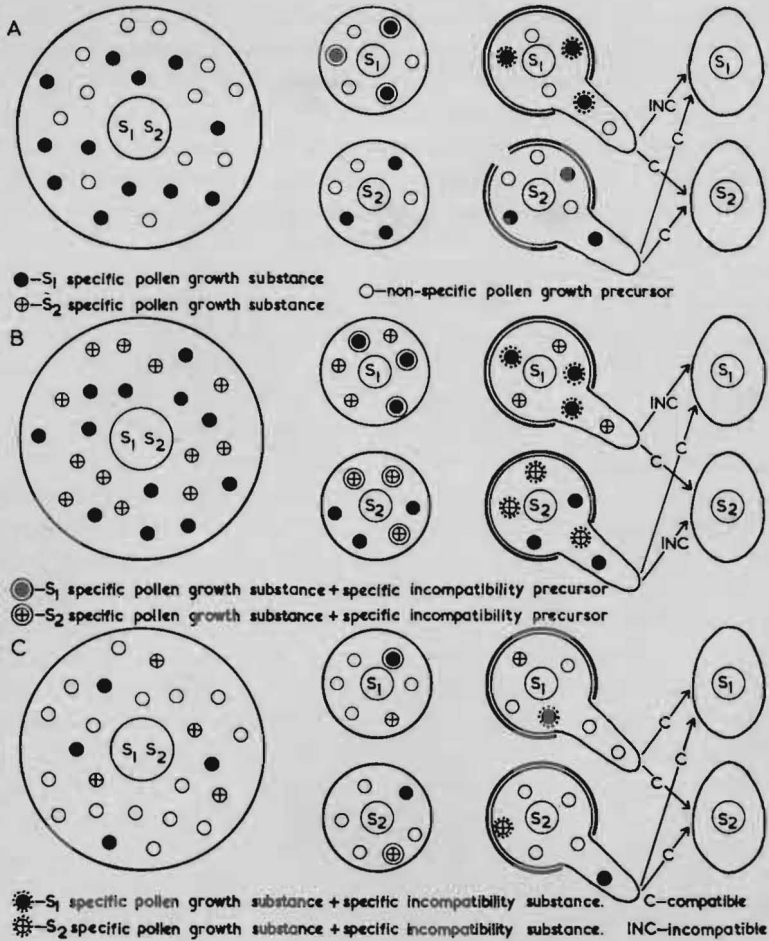


FIGURE 1. The suggested sequence of events regarding the physiology of S gene action in *T. cacao*. There may occur dominance interaction (A), independent action (B), or competitive interaction (C) between S alleles. For details, see text.

and in embryo-sacs in the female, (4) growth of all pollen-tubes down the style and into the embryo-sacs because of lack of incompatibility substances in the sporophytic, stylar and ovarian, tissues and (5) incompatible reaction between the pollen-tubes and embryo-sacs possessing the same dominant S allele, for which specific incompatibility substances are present and compatible reaction between the pollen-tubes and embryo-sacs either of which have the recessive S allele and therefore lack the specific incompatibility substances. Thus, in incompatible pollinations normal fertilization occurs in embryo-sacs containing the recessive S allele but the growth of the embryo is later stopped, presumably by the diffusion of toxic substances from the incompatible embryo-sacs of the same ovary. These processes are controlled by the S gene complex alone. Nevertheless, to explain the self-incompatibility of the hybrid between members of two different self-compatible populations, Cope found it necessary to postulate two independent loci; this seems unwarranted.

Three kinds of *S* allele interactions are known in a diploid cell: (1) *dominance* of one allele over the other; (2) *independent* action of both alleles and (3) interaction leading to *competition* between the alleles neither of which are able to express themselves fully.

The first two interactions have already been demonstrated in *T. cacao*. It is suggested here that competitive interaction, would explain the self-compatibility of certain populations and the self-incompatibility of the hybrids between members of two such different populations. A tree having two competitively interacting *S* alleles would not produce enough specific pollen growth substance for either of the two alleles and would thus be fully self-compatible. A cross between two such unrelated trees would produce progeny all of which may be self-incompatible. Crosses are also possible in which the proportion of self-compatible to self-incompatible plants in the progeny may be 3:1, 1:1 or 1:3. With this hypothesis, the available data are adequately explained on the basis of *S* alleles alone.

The incompatibility system in *T. cacao*, though functionally sporophytic, lies between sporophytic and gametophytic systems and is theoretically still close to the latter, from which it probably originated through interspecific hybridization. This course of evolution is suggested by the results obtained in *Oenothera* hybrids. Under the influence of some alien cytoplasm, particularly that of a self-compatible species, the activities of certain units of an *S* allele complex, from a gametophytic species, may begin precociously (premeiotically), thereby making the *S* allele action partly sporophytic and partly gametophytic. In *T. cacao* the interaction between *S* allele complexes, which leads to sporophytic reaction, is limited to the units controlling the production of specific growth substances, there being no interaction between the units controlling the production of specific incompatibility precursors, which remain strictly gametophytic in action. This leads to simple gametophytic reaction of the pollen-tubes and embryo-sacs.

In the probable evolution of a fully sporophytic system from a gametophytic one, the competitive interaction between *S* alleles, which bring about self-compatibility, must have been eliminated by selection (Pandey, 1960). The occurrence of fully operative *S* alleles, showing competitive interaction in self-compatible populations of *T. cacao*, indicates that the present system of self-incompatibility is of comparatively recent origin.

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OBSERVATIONS ON THE SEXUAL BEHAVIOR OF *DROSOPHILA*
EQUINOXIALIS AND *DROSOPHILA PROSALTANS*

Several works (Hoenigsberg and Koref Santibanez, 1959a, b, c) have shown that incipient sexual isolation can be observed at the sensorial level. Some aspects of courtship discrimination may result in preferences which in the end may yield observable mating differences (Hoenigsberg and Koref Santibanez, 1959c).

With the male choice method described by others (Dobzhansky and Mayr, 1944; Spieth, 1949) and by the method of employing silver paint on the thorax to distinguish the females more readily (Knight, Robertson and Waddington, 1956), the authors studied the possible sensorial discrimination of *D. equinoxialis* and *D. prosaltans*—both from the American tropics.

D. equinoxialis were obtained from seven natural populations, representing four geographical areas:

- (1) Cuba: one strain from Cienfuegos (C 25) and one from Baracoa (C 101).
- (2) Costa Rica: both strains came from Santa Ana but were caught at different times (CR 9 A and CR 9 B).
- (3) Colombia: one strain from Santa Marta (Co 26 F) and one from Bucaramanga (Co 26 G).
- (4) Brazil: only one strain was used (Br). It came from Tefe, Amazonas.

The specimens of *D. prosaltans* originated in three areas:

- (1) Mexico: one strain from Huichenhugan (Me).
- (2) Trinidad: one strain from Sangre Grande (Tr).
- (3) Brazil: one strain from Cantareira, Sao Paulo (SP).

All flies were kept at constant temperature (23°C) on laboratory corn meal and sugar medium. Within a few hours from hatching males and females were separated, to be used in the observations at four days of age. Observations were 30 minutes long as a maximum, but were terminated the moment copulation took place. This note refers to the information gathered from 662 *D. equinoxialis* males and 153 *D. prosaltans* males observed in courtship. Insemination analysis was done on 16 females from two different localities which had been placed in a food vial with eight males from one of these localities. As in courtship analysis, the females of one of the two localities were marked with paint on the thorax to facilitate recognition. Ample time (12 hours) was allowed to permit recovery from etherization. After a period of three hours for *D. equinoxialis* and two hours for *D. prosaltans* (23°C) the females were removed from their food vials and were dissected, using a binocular microscope to examine the ventral receptacle and spermathecae for the presence of sperm. For the insemination study alone 2524 *D. equinoxialis* and 780 *D. prosaltans* females were dissected.

Since a detailed analysis of all the data is not possible in this communication, it is sufficient to mention in passing the general preferential courtship which Colombian males have for the females of their own locality. The same tendency, but with a greater number of examples, was found in the

male Brazilian representatives of *D. equinoxialis*. Courtship is considered as having 15 features which are repeated constantly during the ritual (Spieth, 1952). These elements of courtship are: (1) orientations of the male toward the females, (2) tapping the female with fore tarsi, (3) wing vibration, (4) lickings, (5) kicking by the female, (6) fluttering, (7) circling, (8) acceptance response by opening wings, (9) mounting trial, (10) decamping, (11) wing closure, (12) wing opening as a sign of encouragement, (13) running as a repulsion sign, (14) standing of the female while the male courts her, (15) total duration of the courtship.

TABLE 1

Number of orientations and tappings by *D. equinoxialis* males from Brazil. The + sign indicates preference towards own female; the - sign indicates a preference toward the other female, while ind. refers to males whose direction of courtship was random.

♂	♀♀	Orientations					Tappings						
		n	Own %	Other %	χ^2	P%	n	Own %	Other %	χ^2	P%		
Br	Br, C 25	39	82.0	18.0	8.01	1	+	142	71.8	28.2	13.5	1	+
Br	Br, C 101	34	79.4	20.6	5.38	1	+	137	96.3	3.7	58.8	1	+
Br	Br, CR 9A	52	57.6	42.4	0.61	30-50	ind.	167	55.1	44.9	0.8	30-50	ind.
Br	Br, CR 9B	25	96.0	4.0	10.5	1	+	146	96.5	3.5	63.3	1	+
Br	Br, Co 26F	15	86.6	13.4	4.00	2-5	+	38	100.0	0.0	18.0	1	+
Br	Br, Co 26G	61	80.3	19.7	11.2	1	+	211	88.1	11.9	61.4	1	+

In table 1 we show the differential display of males from Brazil, manifested in distant stimuli (orientations), and in proximal stimuli (tappings) directed toward females from either of two localities. A tendency toward preference of their own kind is consistent in wing vibrations, circlings, courtship time, copulations, lickings and standing position of the females. Only Colombian males of *D. equinoxialis* showed discrimination in courtship, showing preferences not only in proximal stimuli, but in the features of courtship which convey distant stimulation (orientations, wing vibrations, etc.). All the other strains of *D. equinoxialis* showed neither distant nor proximal preferences in courtship with the females from their own choice.

The data obtained by observation of *D. prosaltans* males indicate a clear case of courtship and copulation preference for females from their own locality.

However, there were distinct cases of random choice when the female from outside the male's locality was from Sao Paulo. Orientations, as can be seen in table 2, were less used in discrimination than were the other elements of courtship.

The authors conclude that sensory discriminations which yield preferential courtship and copulations in natural populations do exist and that they favor immiscibility. In nature, where these mechanisms are presumably effective in marginal populations involving contiguous geographical strains,

TABLE 2

Number of orientations and tappings done by *D. prosaltans* from
Trinidad, Mexico, and Sao Paolo.

♂	♀ ♀	Orientations					Tappings						
		n	Own %	Other %	χ^2	P%	n	Own %	Other %	χ^2	P%		
Tr	Tr, Me	136	63.2	36.8	4.76	2-5	+	306	68.3	31.7	28.4	1	+
Tr	Tr, SP	141	67.3	32.7	8.51	1	+	310	59.6	40.4	5.31	1-2	+
Me	Me, Tr	37	78.3	21.7	5.95	1-2	+	158	93.0	7.0	60.8	1	+
Me	Me, SP	92	55.4	44.6	0.54	30-50	ind.	475	77.0	23.0	69.5	1	+
SP	SP, Me	55	54.5	45.5	0.22	50-70	ind.	175	69.1	30.9	12.8	1	+
SP	SP, Tr	199	48.7	51.3	0.06	80	ind.	237	63.4	36.6	10.3	1	+

natural selection may act to develop further the mechanisms of self recognition.

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