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STUDY OF INTER-RELATIONSHIP, PHYLOGENY AND EVOLUTIONARY TENDENCIES IN GENUS *OCIMUM*

M. K. KHOSLA

Regional Research Laboratory, Jammu Tawi 180001

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ABSTRACT

Genus *Ocimum* is an aromatic herbaceous plant used in perfumery, flavouring and pharmaceutical products. A detailed study on inter-relationship, phylogeny and evolutionary aspects in various *Ocimum* species, their different morphotypes and geographical races was carried out based on morphology, distribution, cytogenetical parameters, hybridization, essential oils, breeding, and key diagnostic characters.

Key words: *Ocimum*, inter-relationship, phylogeny and evolution.

The usual concept of species is that they are groups of intermating natural populations and reproductively isolated from other such groups [1, 2]. The concept is generally known as the biological species concept. With the recognition and appreciation of the dynamic variation within the species, it has become obligatory to evaluate the pattern of interspecific variation in order to determine the phylogenetic status of a species. To understand the phylogeny of a species, it is necessary to evaluate the data on its morphology, cytology, genetics, ecology, biochemical, geographical and other aspects [3–7].

Distribution. The genus *Ocimum* (family Lamiaceae) has tropical distribution, with nearly two-thirds of the 160 species [8] reported from Africa and the remaining one-third from Asia and America. Nine species have been identified from India mainly distributed in the tropical areas. On the basis of data on geographic distribution, it has been suggested that there are at least three centres of diversity for this genus. The three centres are 1) tropical and subtropical regions of Africa, 2) tropical Asia, and 3) tropical part of America (Brazil). Tropical Africa is considered to be the primary centre of origin, since maximum diversity of the genus is reported from there [9]. In no other single region, the number of species reported exceeds fifteen. This large variation in the number of species in tropical Africa is indicative on an active evolutionary changes taking place in that region. There are reports of several chemical races with broad geographical range in the "Basilicum" group of *Ocimum*, which includes *O. canum* (two chemotypes), *O. basilicum* (eight chemotypes), *O. americanum* (two chametoypes), and *O. kilimandscharicum* (one chemotype) [10]. The distribution of the species investigated by us under the "Sanctum" group, which includes *O. sanctum* (two morphotypes: green & purple), *O. gratissimum* (three geographical races; Jammu, USA & Kerala), *O. viride, O. suave* and *O. carnosum* shows that only *O. sanctum* and *O. gratissimum* are indigenous to India while the remaining three species, *O. viride, O. suave* and *O. carnosum,* are recent introductions. *O. sanctum* is found only under cultivation and has no wild relatives. *O. gratissimum,* with the exception of Kerala, where it was found in wild state, is mostly cultivated in India or occurs as an escape. *O. gratissimum,* also found in Kerala, appears to be endemic to that region. The natural distribution of this species in Africa is only in wild. Different chemical races and varieties of *O. gratissimum* has been reported from Africa [11]. It is possible that tropical Africa is the original home of *O. gratissimum* from where it spread to Asia. *O. carnosum* is distributed in central America.

Morphology. A comparison of the morphological characters of the species from the "Sanctum" group with those belonging to the "Basilicum" group revealed many interesting features of taxonomic and phylogenetic importance (Table 1). Based on these data, one may broadly agree with the division of genus *Ocimum* into two groups. The species under group I (Basilicum) are predominantly herbaceous, annual, with petiolate bracts, conspicuous flowers and are comparatively long lived, protandrous. The lower lip of the fruiting calyx is narrowed into tooth-like bristle. Seeds ellipsoid and mucilaginous on wetting. On the other hand, species belonging to group II (Sanctum) are predominantly perennial woody undershrubs. Bracts sessile, lower lip of the calyx elongated into bristles or broadened like a flap covering the seed in the calyx tube. From the evolutionary point of view, its annual habit, herbaceous nature, protandrous condition (a mechanism promoting outbreeding), more conspicuous floral parts, and bee pollination in the "Basilicum" its members can be considered to be more advanced than the species belonging to the "Sanctum" group. Flower in the "Sanctum" group is reduced as compared to "Basilicum" group and not much attractive to insects. It is, therefore, possible that the reduced flower in the "Sanctum" group evolved from the large-flowered species in the ancestors of genus *Ocimum.* Studies on breeding behaviour also show that species under the "Basilicum" group are better outbreeders than those from the "Sanctum" group.

The two morphological types of *O. sanctum* (both occurring in India) are identified on the basis of their colour as green and purple. The purple colour seems to be a recessive character. It is possible that the purple trait originated from green through mutation and selected by man. The differences in their vegetative characters such as plant height, petiole length, leaf size and internodal length are of minor nature to warrant their separation into subspecies.

An investigation of the three races of *O. gratissimum* reveals the mode of speciation due to large geographical isolation. While the materials collected from Jammu and USA are identical and agree completely with the description of *O. gratissimum*, the third taxon collected from Kerala showed some differences [12]. General appearance of the taxon (Kerala race) suggested its relationship to *O. gratissimum* but could be easily distinguished from the other two races by more slender habit, nature of leaf (which is more or less glabrous, small and subobovate), length of petiole and flower arrangement (compactly arranged whorls).

O. gratissimum and *O. viride* show some resemblance in their external morphology. The major differences separating these two species are the leaf texture: 1) pubescent in *O. gratissimum*, coriaceous in *O. viride*; and 2) flower colour: brownish green in *O. viride* and green yellow to creamish white in *O. gratissimum*. The calyx and filaments are comparatively smaller in *O. viride* as compared to *O. gratissimum*. The similarity of morphological characters of these two species suggests their close relationships. *O. suave*, another species studied, also shows resemblance in some morphological characters to *O. gratissimum* and *O. viride*, especially floral characters like corolla size, length of stamens, style and fruiting calyx. But the vegetative characters of *O. suave* are quite different from these of *O. viride* and *O. gratissimum*. *O. carnosum* possesses many characters of both "Bacilicum" and "Sanctum" groups. In respect of leaf size, texture and floral characters, it resembles *O. gratissimum* and other species of the "Sanctum" group. But in seed colour, shape (black and ellipsoid) and mucilaginous nature of seed, it resembles the species of the "Bacilicum" group. Out of the five *Ocimum* species investigated, *O. gratissimum*, *O. viride* and *O. suave* show taxonomic and genetic relationships, suggesting their origin from a common ancestor. On the other hand, *O. sanctum* and *O. carnosum* have no relation or resemblance to each other or with the other three species of *Ocimum*. These two species appear to have originated separately from a common ancestral stock with other three species which got differentiated later. From evolutionary point of view, the semiwoody and biennial *O. sanctum* appears to be more advanced as compared to *O. gratissimum*, *O. viride* and *O. suave*. *O. carnosum* also seems to be more advanced than the remaining three species under investigation.

Cytology. Cytological studies provided valuable informations regarding inter-relationships and evolutionary trends in the genus *Ocimum*. A survey of the chromosome numbers recorded (Table 2) in genus *Ocimum* clearly suggests existance of more than two basic numbers. The different haploid chromosome numbers so for recorded are 12, 13, 16, 20, 24, 32, 36 and 38, indicating clearly that in *Ocimum*, the numerical variation by way of aneuploidy and polyploidy have played an important role in the diversification of the genus as well as evolution of a series of new basic chromosome numbers. It has been experimentally demonstrated that new *Ocimum* species can be created through allopolyploidy while resynthesizing the hexaploid *O. americanum* from its putative parents

Table 1. Comparative morphology of *Ocimum* species belonging to the "Basilicum" and "Sanctum" groups

Species	Habit	Leaf	Bract	Flower	Fruiting calyx	Seeds	Essential oil constituents	Chromosome No. (n)
Basilicum group:	Herbaceous, annual	Glabrous or pubescent	Petiolate	Conspicuous, protandrous	Lower lip of the fruiting calyx-lobes elongated to bristles	Mucilaginous when wetted	Predominantly terpenes, rarely with phenols	
O. basilicum L.	"	Glabrous	"	" Corolla 4-12 mm long, white	"	"	"	24
O. americanum L.	"	Pubescent	"	" Corolla 6-8 mm long, white	"	"	"	36
Ocimum sps. No. 7 (new species)	"	"	"	" Corolla and anther, pinkish red	"	"	"	30
O. kilimandscharicum Guerke	"	Pubescent	"	" Corolla 8–9 mm long	" Small, 3.5–5.5 mm long	"	"	38
O. canum Sims	"	Pubescent	"	" Corolla 4.5–5.5 mm long	"	"	"	12, 13
Sanctum group:	Perennial woody under shrub	Glabrous or pubescent	Sessile	Less conspicuous, autogamous	Lower lip of fruiting calyx broader and not elongated to bristle-like teeth	Brownish, black-globose, subglobose, nonmucilaginous when wetted	Phenols with small amount of terpenes	
O. sanctum L.	Annual or biennial	Small pubescent	"	"	Fruiting calyx small, 4.0–5.5 mm long	"	"	16

(Contd.)

Table 1 (contd.)

Species	Habit	Leaf	Bract	Flower	Fruiting calyx	Seeds	Essential oil constituents	Chromosome No. (n)
O. gratissimum L.	Perennial	Large, pubescent to nearly glabrate	"	"	Fruiting calyx large, lobes strongly nerved, 5.0–7.8 mm long	"	"	20
O. viride Willd.		Large, nearly pubescent, coriaceous	" lobes strongly nerved, 5.5–7.0 mm long	"	Fruiting calyx, large amount of terpenes	"	Phenols with small	20
O. suave Willd.	"	Small, highly pubescent	"	"	Fruiting calyx 5.5–7.0 mm large, long alcohols	"	Predominantly sesquiterpene	24
O. carnosum Lk. et Otto.	"	Large, glabrate	"	"	Fruting calyx large, 6.9–9.0 mm long	Ellipsoid and slightly slimy	Predominantly phenols	32

O. canum (diploid) and *O. basilicum* (tetraploid) [13]. The present study also clearly demonstrates that polyploidy, especially allopolyploidy and aneuploidy (both ascending and descending), have played a major role in the evolution of new *Ocimum* species. It is possible that *O. gratissimum* and *O. viride* with 2n = 40 chromosomes each have evolved as allopolyploids from taxa with 2n = 40, and *O. suave* with 2n = 48 chromosomes likewise evolved from taxa with 2n = 24 chromosomes. The chromosome pairing in the interspecific hybrids between *O. gratissimum, O. viride* and *O. suave* (Table 3) also supports its origin as allopolyploid. Since majority of *Ocimum* species are still not investigated cytologically, it is possible that the diploid relatives of the ancestors of many polyploid species presently investigated may still be available in the thick rain forests of tropical Africa where maximum diversity of the genus exists. Several factors like genic changes, polyploidy (most likely allopolyploidy), hybridization etc. appear to have played a significant role in species differentiation in genus *Ocimum*. Such instances have also been reported in the genus *Argemone* [14].

The present study shows that *O. gratissimum* and *O. viride* with the same chromosome number and producing partially fertile F_1 hybrids

Table 2. Chromosome numbers of *Ocimum* species

Species	Chromosome number	Reference	
O. canum Sims	12	Morton [11]	
		Pushpangadan et al.	1975
	13	Pushpangadan et al.	1975
	32	Golubinski	1937
		Sanjappa	1979
	64	Golubinski	1937
	32+0–4B	Vij and Kashyap	1976
O. basilicum L.	24	Vaarama	1947, 1954
		Morton [11]	
		Pushpangadan et al.	1975
		Sanjappa	1979
O. americanum L.	36	Sobti et al.	1975
O. kilimandscharicum Guerke	38	Choudhary et al.	1955
		Kumar et al.	1957
		Bose & Choudhary	1959, 1962
		Mehra and Gill	1972
		Pushpangadan et al.	1975
Unidentified *Ocimum* species	30	Sobti and Pushpangadan [13]	
O. sanctum L.	16	Golubinski	1937
		Mehra and Gill	1972
		Khosla and Sobti	1985
	16+0–3B	Vig and Kashyap	1976
		Golubinski	1938
		Sanjappa	1979
O. gratissimum L.	20	Golubinski	1938
		Morton	1962
		Getsadze	1975
		Khosla and Sobti [24]	1985
	24	Golubinski	1938
		Morton [11]	
	32	Golubinski	1938
		Mitra and Dutta	1967
O. viride Willd.	19	Singh	1978
	20	Khosla and Sobti [24]	
O. suave Willd.	16	De Wet	1958
	32	Morton [11]	
	24	Khosla and Sobti [24]	
O. carnosum Lk. et Otto.	24	Singh	1978
	32	Khosla and Sobti [24]	
O. irvinei J.K.M.	16	Morton [11]	
		Sobti and Pushpangadan [10]	
O. adscendens Willd.	32	Sanjappa	1979

are closer and evolved as two different species more recently and are still in the process of forming complete isolation barriers. Being partially fertile, they are still at a stage of evolution where the gene flow can take place from one species to other species as reported in genera like *Clarkia* [15–20], *Phlox* [21], *Howarthia* and *Aloineae* [22, 23].

Karyomorphological investigations of species of the "Sanctum" group [24], when compared with the Karyomorphological studies of species belonging to the "Basilicum" group revealed many interesting features. The length of chromosomes and gross appearance of karyotypes show a general resemblance in all the species belonging to both the groups. Chromosomes of the species belonging to "Sanctum" group are shorter. The illustrations examined in the present work as well as in earlier studies suggest that genus *Ocimum* displays a wide range of variation in chromosome number and there is no typical karyotype which could be singled out as the basic karyotype of the genus. But a careful analysis of the chromosome complements of different species, shows that chromosomes of the species of "Sanctum" group can be divided into five groups on the basis of their total length. All the species investigated have more than one set of chromosomes and each set consists of predominantly, 8, 10, 12 chromosomes or multiples of these numbers. These chromosome groups in sets of 8, 10 and 12 provide additional support to the polybasic origin of "Sanctum" group. It appears that each chromosomal set in these groups is the original genome with $x = 6$ or 8 chromosomes, and the species studied presently evolved as allopolyploids from these ancestral stocks.

Meiotic pairing in the interspecific hybrids of *Ocimum* have also provided important information. The presence of average ten bivalents in the F_1 hybrids involving *O. gratissimum*, *O. viride* and *O. suave* suggests that ten chromosomes from each of these species are homologous. This indicates that these species share a common genome with $x=10$ chromosomes. This also supports the assumption based on earlier karyomorphological studies. Heterologous and partial pairing suggests segmental homology. Occasional bridge formation observed in interspecific crosses and F_1 hybrids between different geographical races is a sufficient proof that chromosomal translocations and inversions have played an important role in karyomorphological evolution, leading to speciation in genus *Ocimum*.

Essential oils. Studies on the essential oil composition in different species of the "Sanctum" group [25] showed that essential oils of these species contain predominantly phenols (eugenol, isoeugenol, methyl eugenol, elemicin), monoterpene phenol (thymol) and sesquiterpenes as major essential oil constituents, and the monoterpene compounds are mostly found as minor constituents. It may be pointed out that the species belonging to "Basilicum" group have predominantly monoterpenes (citral, linalool, geraniol and camphor) whereas phenolic and sesquiterpene compounds are found only as minor constituents. This suggests that species belonging to both groups have genes for the synthesis of phenol, monoterpenes and sesquiterpene compounds. Species belonging to the "Basilicum" group have evolved to produce terpene-rich compounds whereas the species of "Sanctum" group have evolved for phenolic and sesquiterpene-rich oils. It is pertinent to note that species of the "Sanctum" group are woody and perennial whereas those from "Basilicum" group are annuals. The synthesis of phenols in the "Sanctum" group and monoterpenes in the "Basilicum" group may be related with the perennial woody habit in the former and annual herbaceous habit in the latter [26, 27].

Each species is a reproductive community. It consists of populations, each with an integrated gene pool. Each species constitutes the stage of evolution when a major genetic system becomes closed and loses its ability for interbreeding and fusing with other such systems. The number of populations may interbred among themselves or with closely related populations of the same species and thus maintain a certain degree of genetic variability in the species which is essential for its further evolution. This is specially true for the basically crossbreeding species. The crossbreeding species have retained, to a certain extent, their ability to outbreed with populations of their closely allied varieties or species [28].

Table 3. Chromosome pairing behaviour at meiosis in the hybrids of *Ocimum* species

Meiotic stage	Diakinesis-Metaphase-I						Anaphase I			Anaphase II			
	I	II	III	IV	V	VI	segregation	laggards	restitution	No. of chromosome	No. of micronuclei	No. of laggards	No. of restitution
O. gratissimum (2n=40, USA) x *O. gratissimum* (2n=40, Kerala)	4–20 (9.5)	10–18 (14.93)	0–1 (0.1)	0–1 (0.03)		0–1 (003)	18+16–22+18 (20.07+18.15)	0–6 (1.78)	—	3–4 (3.86)	0–1 (0.06)	0–4 (0.76)	0–1 (0.14)
O. gratissimum (2n=40, Kerala) x *O. gratissimum* (2n=40, USA)	4–18 (10.40)	10–18 (14.40)	0–1 (0.39)	0–1 (0.40)	—	—	17+17–23+17 (20.20+18.50)	0–6 (1.30)	—	3–4 (3.78)	0–1 (0.12)	0–3 (0.86)	0–1 (0.22)
O. gratissimum (2n=40, USA) x *O. viride* (2n=40)	14–34 (22.64)	3–13 (8.32)	0–1 (0.24)	—	—	—	14+12–25+10 (19.67+15.95)	0–14 (4.38)	0–1 (0.02)	2–7 (3.88)	0–3 (0.40)	0–3 (0.42)	0– (0.46)
O. viride (2n=40) x *O. gratissimum* (2n=40, USA)	10–26 (17.23)	7–15 (11.33)	0–1 (0.03)	—	—	—	13+13–22+12 (18.31+14.66)	0–14 (7.03)	0–1 (0.03)	2–6 (3.92)	0–6 (0.30)	0–7 (1.48)	0–1 (0.30)
O. viride (2n=40) x *O. suave* (2n=48)	6–36 (22.11)	4–19 (10.71)	0–1 (0.06)	0–1 (0.06)	—	—	16+14–24+20 (21.47+18.76)	0–14 (3.76)	0–1 (0.03)	1–5 (3.67)	0–2 (0.12)	0–8 (2.54)	0–1 (0.30)
O. suave (2n=48) x *O. viride* (2n=40)	10–30 (20.47)	7–17 (11.2)	0–1 (0.2)	0–1 (0.13)	—	—	17+15–24+20 (20.56+16.28)	0–12 (7.15)	0–1 (0.03)	1–7 (3.68)	0–4 (0.03)	0–8 (2.98)	0–1 (0.46)

Note. Mean values are given in parentheses.

Breeding. The breeding system is an important factor in determining the genetic structure and evolutionary dynamics of a population [29, 30]. The *Ocimum* species are basically crosspollinated. Most of the species interbreed among themselves or with closely allied populations of the same species and thus maintain a certain amount of genetic variability in the population which is essential for further evolution of the species. These crossbreeding species have retained their ability to interbreed with populations of their closely allied varieties or species. The breeding system is, thus, an important factor in determining the genetic structure and evolutionary dynamism of a population. Hybridization occurs between populations of the same *Ocimum* species or between different morphological and chemical types, or between different varieties of the same species. Further, outbreeding of the species with other species also occurs, which often leads to a large scale introgression of many characters in the allied species as well as provides new material for evolution. The present study provides enough evidence to support this view.

The F_2 population raised from the partially fertile selfed F_1 hybrids involving *O. gratissimum* and *O. viride* included several new morphological, cytological and chemical variants with the chromosome number ranging from 40 to 80 [31], which were outside the usual ranges of parental populations. A complete reshuffling of morphological, cytological and chemical characters of the two parents occured, resulting in the development of new forms, some of which had less chance to survive while some others had great potential for survival in more diverse conditions. Plants with chromosome numbers $2n = 40, 42, 44, 45, 48$ and 80 survived to maturity and flowered while many others died before maturity. These high surviving types have every chance to spread to new ecological nitches. Some of the F_2 plants had the same chromosome numbers $2n = 40$, but differed in the morphological characters transmitted from the two parents, while some plants even had a few characters not present in the parents. One such new variant, a gigantic plant, was found to be amphidiploid; similar to the amphidiploid synthesized experimentally from F_1 hybrids [32]. A few plants had morphological features similar to *O. viride* but had eugenol as the major essential oil constituent, while other plants with *O. gratissimum* characters had thymol in oil. Some plants with aneuploid chromosome numbers ($2n = 42, 44, 45$) showed high fertility. Many workers [33–36] have suggested catalytic effect of hybridization on the biological evolution of two species. In the first place, genetic recombination in the wholly and partially fertile progeny of hybrids gives rise to large quantitative increase in the size of gene pool. Such evolutionary jumps have been reported in a number of plant genera like *Elymus, Potentialla, Linanthus, Galeopsis, Gilia, Nicotiana* [37, 38].

The *Ocimum* species mostly evolved perennial to annual habit. Both gradual and abrupt speciation are operative in the genus. Gradual speciation [39] due to multiple genetic changes without major chromosome rearrangements is operating in the Kerala race of *O. gratissimum.* Barrier in gene exchange or isolation from other races of this species appear to have appeared gradually in this taxon through accumulation of mutations or minor chromosomal rearrangements (as is evident from the meiotic chromosomal rearrangements (as is evident from the meiotic behaviour of chromosomes in the F_1 hybrids of the Kerala race with the other two races) and long geographic isolations. In this case, the reproductive isolation due to chromosomal as well as genetic changes has developed which may result in evolving a subspecies and ultimately a new species. Evolution leading to such speciation is considered to be a general process that includes successive stages from the formation of partially detectable ecological or geographic races to eco-geographical differentiation readily recognisable at the level of subspecies. Subsequent development of reproductive barriers leads to differentiation at the level of species [40–43].

The origin of *O. americanum* from *O. canum* and *O. basilicum*, an experimentally demonstrated [44], is a clear evidence of abrupt speciation being operative in the evolution of *Ocimum* species.

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REFERENCES

1. E. Mayer. 1970. Population, Species and Evolution. Harvard Univ. Press, Cambridge.

2. L. V. Valen. 1976. Ecological species, multispecies and oaks. Taxon, **25**(2/3): 233–239.

3. H. O. Lam. 1948. Classification and the new morphology. Acta Biotheor., **8**: 107–158.

4. A. J. Eames. 1951. Again, the new morphology. New Phytol., **50**: 17–35.

5. A. J. Eames. 1957. Some aspects of progress in plant morphology during the past fifty years. Amer. J. Bot., **44**: 100–104.

6. K. K. Sporne. 1959. On the phylogenetic classification of plants. Amer. J. Bot., **46**: 385–394.

7. Y. Ogura. 1964. Comparative morphology and classification of plants. Phytomorphology, **14**: 240–247.

8. J. C. Willis. 1966. A Dictionary of Flowering Plants and Ferns (revised 7th edn.). Cambridge Univ. Press, London.

9. M. K. Khosla. 1981. Cytogenetical Investigations in the Genus *Ocimum* with Special Reference to the "Sanctum" group. Ph. D. thesis, University of Jammu, Jammu.

10. S. N. Sobti and P. Pushpangadan. 1977. Cytotaxonomical studies in genus *Ocimum*. *In*: Taxonomy, Cytogenetics, Cytotaxonomy of Plants (ed. S. S. Bir). Kalyani Publishers, New Delhi: 373–377.

11. J. K. Morton. 1962. Cytotaxonomic studies on West African Labiatae. J. Linn. Soc. Bot., **58**(372): 231.

12. M. K. Khosla and S. N. Sobti. 1984. Hybridization between different geographical races of *Ocimum gratissimum* L. **27**(3): 156–159.

13. S. N. Sobti and P. Pushpangadan. 1977. Studies in genus *Ocimum*. Cytogenetics, breeding and production of new strains of economic importance. *In*: Cultivation and Utilization of Medicinal and Aromatic Plants (eds. Atal and Kapoor). Regional Research Laboratory, Jammu: 273–286.

14. C. P. Malik. 1974. Cytogenetical evolution and speciation in *Argemone*. *In*: Advancing Frontiers in Cytogenetics (ed. P. Kachroo). Hindustan Publishing Corporation, Delhi.

15. H. Lewis. 1966. Speciation in flowering plants. Science, **152**: 167–172.

16. E. Small. 1971. The evolution of reproductive isolation in *Clarkia* Section Myxocarpa. Evolution, **25**(2): 330–346.

17. A. F. Hameed and R. Snow. 1972. The origin of the allotetraploid in *Clarkia gracilin*. Evolution, **26**(1): 340–345.

18. B. Bruce, L. L. Eaton and P. H. Raven. 1973. *Clarkia rubicunda*. A model of plant evolution in semiarid regions. Evolution, **27**(3): 505–517.

19. L. D. Gottleib. 1973. Enzyme differentiation and phylogeny in *Clarkia franciscana*. Evolution, **27**(2): 205–214.

20. L. D. Gottleib. 1974. Genetic confirmation of the origin of *Clarkia lingulata*. Evolution, **28**(2): 244–250.

21. D. A. Levin. 1970. The exploitation of pollinators by species and hybrids of *Phlox*. Evolution, **24**(2): 367–377.

22. A. K. Sharma and K. B. Datta. 1962. An investigation on the cytotypes of *Howarthia*. Genet. Iber., **14**: 131–155.

23. A. K. Sharma and R. Mallick. 1965. Inter-relationship and evolution of the tribe Aloineal as reflected by cytology. J. Genet., **59**: 20–47.

24. M. K. Khosla and S. N. Sobti. 1985. Karyomorphological studies in genus *Ocimum*. II. "Sanctum group". Cytologia, **50**(2): 253–263.

25. M. K. Khosla, S. N. Sobti and C. K. Atal. 1985. Genetic studies on the inheritance pattern of different essential oil constituents of *Ocimum* species. Indian Perfumer, **29**(3–4): 151–160.

26. A. Love. 1964. The evolutionary framework of the biological species concept. Genetics Today. Proceedings of the XI International Congress of Genetics: 404–415.

27. A. Love. 1964. The biological species concept and its evolutionary structure. Evolution, **13**(2): 33–50.

28. H. I. Oka and M. Morishima. 1967. Variation in the breeding systems of a wild rice, *Oryza perennis*. Evolution, **21**: 249–258.

29. Y. E. Chu, M. Morishima and H. I. Oka. 1967. Reproductive barriers distributed in cultivated rice species and their relatives. Japan J. Genet., **44**: 207–223.

30. Y. E. Chu. 1972. Genetic basis, classification and origin of reproductive barriers in *Oryza* species. Bot. Bull. Acad. Sin., **13**: 47–66.

31. M. K. Khosla. 1988. Cytomorphological study of F_2 variants of F_1 hybrids of *Ocimum gratissimum* L. (2n=40) and *O. viride* Willd. (2n=40). Cytologia, **53**(3): 561–570.

32. M. K. Khsola and S. N. Sobti. 1986. Cytogenetic studies in genus *Ocimum*: interspecific hybrids and induced amphiploids of *O. gratissimum* L. (2n=40) x *O. viride* Willd. (2n=40). Cytologia, **51**(1): 225–234.

33. V. Grant. 1963. The Origin and Adaptations. Columbia Univ. Press, New York.

34. V. Grant. 1966. The origin of new species of *Gilia* in a hybridization experiments. Genetics, **54**: 1189–1199.

35. E. Mayer. 1963. Animal Species and Evolution. Harvard Univ. Press, Cambridge.

36. G. L. Stebbins. 1966. Chromosomal variation and evolution. Science, **152**: 1463–1469.

37. G. L. Stebbins. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.

38. G. L. Stebbins. 1971. Chromosomal Evaluation in Higher Plants. Edward Arnold Ltd., London.

39. M. J. D. White. 1968. Models of speciation. Science, **159**: 1065–1070.

40. C. M. Rick. 1963. Barrier to interbreeding in *Lycopersicon esculentum*. Evolution, **17**: 216–232.

41. V. Grant and K. A. Grant. 1965. Flower Pollination in the *Phlox* Family. Columbia Univ. Press, New York.

42. H. Lewis. 1966. Speciation in flowering plants. Science, **152**: 167–172.

43. P. Lagendre and V. P. Court. 1969. A mathematical model for the entities species and genus. Taxon, **18**(3): 245–256.

44. P. Pushpangadan and S. N. Sobti. 1982. Cytogenetical studies in genus *Ocimum*. 1. Origin of *O. americanum* cytotaxonomical and experimental proof. Cytologia, **47**: 575–583.