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FLORAL MORPHS AND MODE OF POLLINATION IN *OXALIS CORNICULATA* L., FROM PAKISTAN

RUBINA ABID

Department of Botany,
University of Karachi, Karachi, Karachi-75270, Pakistan.

Abstract

Homostylous populations of *Oxalis corniculata* L., are recorded from Pakistan (stigmas at the level of long stamens) rarely mixed with semihomostylous flowers (stigmas slightly below the long stamens) or flowers with stigmas at the level of short stamens. A variety of insects visited the flowers in search of nectar and pollen but insects did not give preference to any morph as all the insects were interested either in nectar only or nectar and pollen. Breeding experiments and pollen-ovule ratio indicated the facultative autogamous nature of the taxon which was also supported by the higher fruit set in selfing compared to the crossing.

Introduction

The genus *Oxalis* L., belongs to the family Oxalidaceae with about 500 species, distributed in America, Africa, Europe and Asia (Lourteig, 2000; Mabberley, 2008). *Oxalis corniculata* is a quite common weed, spread all over Pakistan (Nasir, 1971). The species is considered heterostylous with different floral forms i.e., having different lengths of styles in relation to the length of stamens (Faegri & Pijl, 1980). Previously reproduction and style length polymorphism in *Oxalis corniculata* was studied by various workers (Ornduff, 1972; Lovett Doust *et al*., 1981; Hiroyuki *et al*., 1995) and correlated the mode of reproduction with different floral morphs. The present studies were carried out for determining the mode of reproduction and insects behaviour within different morphs of *Oxalis corniculata* from Pakistan.

Materials and Methods

Study sites: The studies were conducted within the vicinity of Karachi i.e., Karachi University campus, Abul Hasan Isphahani Road, Gulshan-e-Iqbal and Malir, while floral phenology was also observed from Karimabad Hunza 2300m, Gilgit Baltistan during 2008-2009.

Floral phenology: Ten young floral buds/population were tagged to determine the phenological changes.

Insects (Visitors): Flower visiting insects were observed for their foraging behaviour. Insects were collected by hand net, chloroformed and observed microscopically for the pollen load. The insects carrying pollen were evaluated as pollinators.

Breeding studies

Pollen–ovule ratio: The flower buds were collected prior to anthesis and pollen ovule ratio was determined by dividing the total number of pollen grains/flower by the total

number of ovules/flower (Cruden, 1977) and following counts were made: (i) Total number of anthers/flower (ii) Total number of pollen grains/anther (iii)Total number of pollen grains/flower (iv) Number of ovaries/flower (iv) Number of ovules/ovary (v) Number of ovules/flower.

Bagging experiments: Following pollination treatments were given in flowering bud stage (N=25).

Control (Open pollination): Buds were tagged and left to study the normal seed set.

Direct autogamy: Buds were bagged without manipulation to determine the self-pollination.

Indirect Autogamy: Buds were hand pollinated and bagged to test the self-pollination.

Apomixis: Buds were emasculated and bagged to test the apomixis.

Geitonogamy: Buds were pollinated by hand with the pollen grains of the other flowers of the same plant and bagged to test the geitonogamy.

Xenogamy: Buds were **c**ross pollinated by hand with the pollen grains of different plants of the same species and bagged to test the xenogomy.

The data was statistically analyzed among different pollination treatments by one-way ANOVA and Bonferoni's Multiple Comparison Test (Anon., 1999).

Observations and Results

Floral phenology: The flowering period of *Oxalis corniculata* ranged from January-May. A young bud took 6-8 days to become a flower. Flowers opening and closing times varied depending on temperature and light. Below or at 20°C flower opened at 10-11am and closed at 3:30-4:30 pm. While, at 30-40°C flowers opened at 9:00-10:00am and closed till 1:30-2:30 pm. Flower survived for about 48 hours after that petals and filaments were shed. Stamens were in two groups, five long and 5 short, stigmas 5 papillate at the level of long stamens or at the level of short stamens or slightly below the long stamens (Semihomostylous) (Fig. 1), anthers and stigma matured just after the anthesis of flower. There were 5 nectaries at the base of ovary and nectar oozed out from the nectaries in the form of droplet around the ovary base.

Pollen-ovule ratio (P/O): The pollen ovule ratio ranged from 93.82-116.92. So the species seemed to be the facultative autogamous (Table1).

Bagging experiments: All the pollination treatments showed the normal fruit set except that of the apomixis. Although fruit production was significantly higher in selfing as compared to the crossing (Table 2; Fig.2).

Insect's behaviour: Different varieties of insects mainly including Diptera and Lepidoptera regularly visited the flowers of *Oxalis conuiculata* for nectar and pollen grains.

Table 1. Pollen ovule ratio of *Oxalis corniculata*.

Floral morphs	Total no. of anthers /flower (short + long)	Average no. of pollen/ anther (short + long)	Average no. of pollen / flower	Average no. of ovaries / flower	Average no. of ovules / ovary	Pollen-ovule ratio
Long styled	5+5	131 + 240= 371 ± 38.87	3710 ± 63.54	1.0	38.66 ± 1.356	97.63 ± 18.28
Mid styled	5+5	106 +198= 304 ± 24.688	3040 ± 42.36	1.0	26.87 ± 2.11	116.92 ± 7.88
Short styled	5+5	83 + 236= 319 ± 13.623	3190 ± 49.28	1.0	34.22 ± 1.32	93.82 ± 14.22

Table 2. Effect of various pollination treatments on fruit set in *Oxalis corniculata*.

One-way ANOVA Bonferoni's Multiple Comparison Test(BMCT)							
Sv	**df**	**Ss**	**Ms**	**F-value**	**Treatments**	**Rank**	**Mean**
Treatments	5	1699.0	3398		Control	1	74a
Error	20	1026.66	51.33	66.19***	Direct autogamy	2	30b
Total	25	2725.66			Indirect autogamy	3	22b
					Geitonogamy	4	12c
					Xenogamy	5	12c

*** = $p<0.001$. Means sharing the same letter do not differ significantly $p>0.05$.

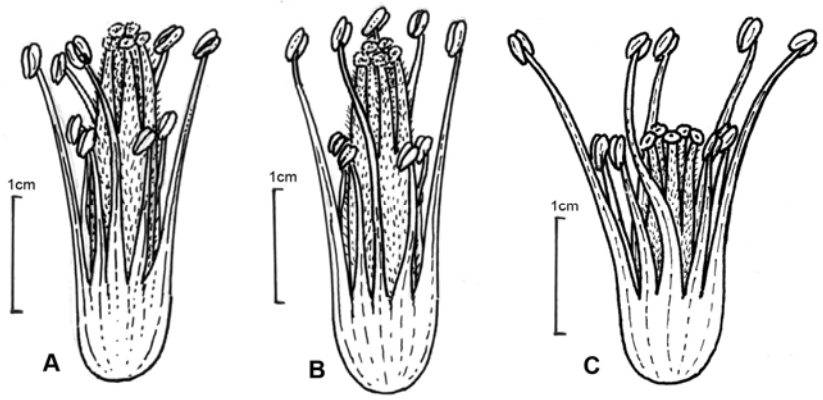

Fig. 1. Different floral morphs showing the style and stamen lengths. A: Stigmas at the level of long stamens; B: Stigmas slightly below the long stamens; C, Stigmas at the level of short stamens.

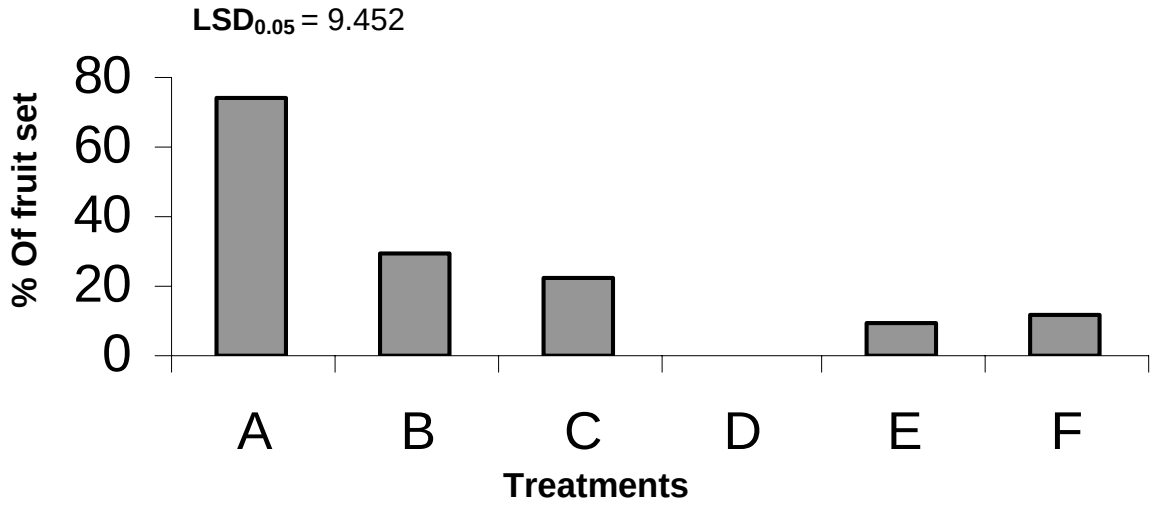

Fig. 2. Percentage of fruit set among different pollination treatments.
A= Control, B = Direct autogamy, C = Indirect autogamy, D = Apomixis, E = Geitonogamy, F = Xenogamy

Lepidoptera (Butterflies) viz., *Pieris rapae, Sylepta deurogata, Lycaena* sp., and *Coletis* sp., were the common visitors (without any pollen load), the activities of all butterflies were same. They alight on petals without touching the reproductive organs and inserted their proboscis into the tube of flower where nectaries were located at the base of ovary to suck the nectar then turn their attention towards the other flower of the same plant or other plant. They were nectar thief and can not be classified as a pollinator (Fig. 4D).

Eristalis sp. (a) started the activity after 20-30 minutes of flower opening. They alighted on petals and acrossing the stamens and stigma insert their proboscis into nectaries and suck the nectar. In this way pollen were also deposited on their legs, thorax and abdomen. After visiting 3-4 flowers they usually depart from the population (Fig. 3A-B).

Eristalis sp. (b) alighted on petals and through its forelegs and proboscis collected the pollen grains from large as well as from small anthers and chewed the pollen grains. Besides this it also tries to suck the nectar by inserting its proboscis in the nectaries. In this way pollen were loaded on abdomen, legs and thorax. It only visits 2 or 3 flowers then stays on the leaf or nearest branch and lick the legs through proboscis to eat or detach the pollen load (Fig. 4A,B).

Eristalis sp. (c) also alighted on petals then collected the pollen grains, from here insect also tried to suck the nectar by inserting its proboscis into nectaries. Then insect moved from one flower to another flower of the same or different plant but preferred those flowers which were getting the direct sun light. During the activity pollen were deposited on legs and ventral body parts (Fig. 4C).

Ceratina sp., alighted on petals and by pushing or griping the reproductive organs it tried to enter its proboscis in the nectaries to suck the nectar in this way a large number of pollen grains get deposited on its body. It also visited 4-5 flowers per population (Fig. 3C-D).

Discussion

Oxalis corniculata is reported to have homostylous populations rarely mixed with heterostylous flowers (stigmas at the level of short stamens) or semihomostylous flowers (stigmas slightly below the long stamens). This is in contrast to the previous findings of Hiroyuki *et al*., (1995) where they reported exclusively homostylous populations or the heterostylous populations without any mixed morphs from Japan. The flowering period of *O. corniculata* ranges from January-May and flowers opening and closing times varied depending on temperature and light. Nectar and pollen are the main rewards for the insects while all the Lepidoptera (Butterflies), *Eristalis* sp., (a) and *Ceratina* sp., visit the flowers only for nectar and similar to that of the previous findings on some members of the family Malvaceae (Gottsberger,1967; Dawar *et al.*,1994; Abid,2006; Abid *et al*., 2010), Lepidoptera were merely visitors as they suck the nectar without touching the reproductive organs. While *Eristalis* sp., (a) and *Ceratina* sp., frequently take part in pollination by transfering the pollen to the stigma of the same or different flowers. However, on the other hand *Eristalis* spp., (b and c) also feed on the pollen. It is also interesting to note that insects did not give preference to any specific morph as all of the insects were interested to get nectar instead of only pollen. Breeding experiments indicated the higher fruit set in selfing than crossing and point out the facultative autogamous nature of the taxon which is also supported by the result of pollen ovule ratio. Although, from Pakistan three different floral morphs of *Oxalis corniculata* are reported but similar to those of the previous findings (Ornduff, 1972; Lovett Doust *etal*., 1981) autogamy seems to be the rule.

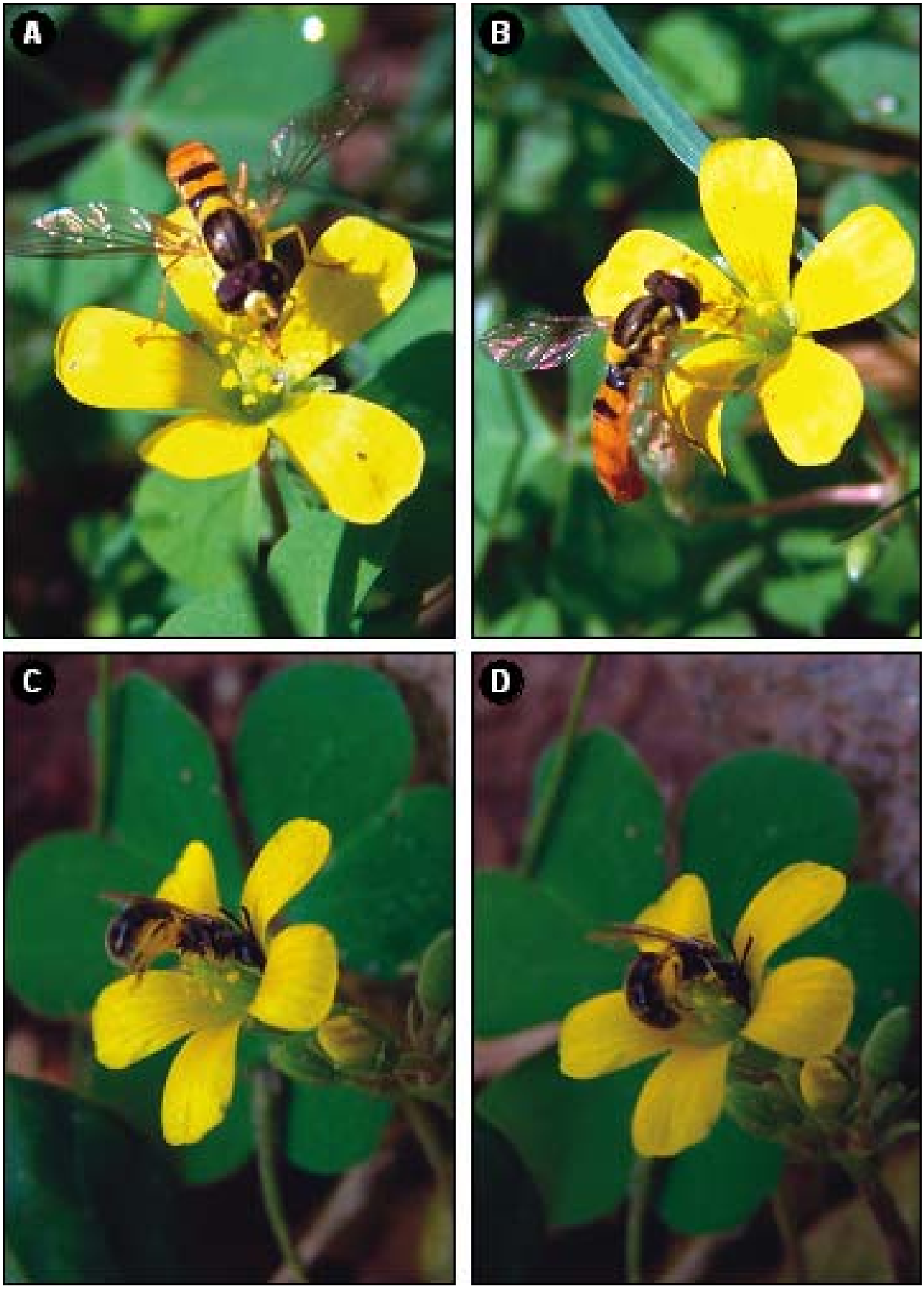

Fig. 3A,B: *Eristalis* sp.a, sucking the nectar; C,D: *Ceratina* sp., sucking the nectar.

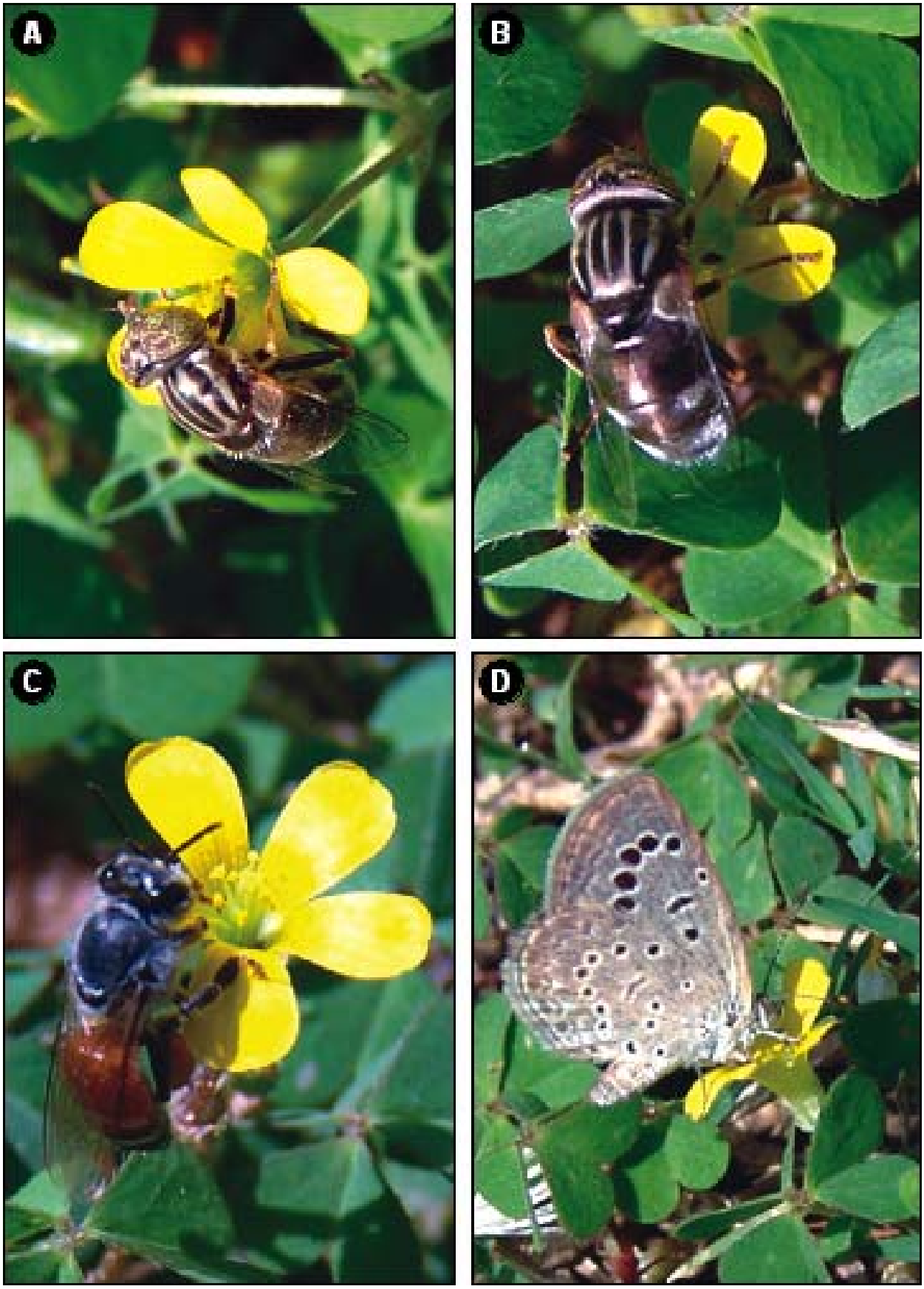

Fig. 4A,B: *Eristalis* sp.b, collecting the pollen grains ; C: *Eristalis* sp.c, collecting the pollen grains; D:*Lycaena* sp., sucking the nectar.

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