Biosystematic studies on the parasitoid *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae) *vis-a-vis* its aphid host

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This thesis is dedicated to my parents and teachers.
Biosystematic studies on the parasitoid *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae) *vis-a-vis* its aphid host

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Introduction
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Abstract
Oilseed Brassicas are among the major oilseed crops cultivated in India and around the world. India produces about 11.3% of the world’s rapeseed-mustard (Damodaram and Hegde, 2002). Among the oilseeds, mustard is one of the major sources of edible oil for human consumption. Lipaphis erysimi (Kaltenbach) (Hemiptera: Aphididae) is the main aphid pest infesting mustard in several areas in India and its infestation by L. erysimi reduces directly both yield and quality of the product and cause up to 83% losses in mustard (Mandal et al., 1994). This species also shows resistance to insecticides in some parts of the world (Wei et al., 1988). Increase in population beyond 9.45 aphids per plant, reduce the seed yield by 59.3 per cent with an economic injury level of 2.04 aphids/plants (Singh and Malik, 1989).

Aphids as pests are known worldwide. Their biological peculiarities along with extensive monoculture and indiscriminate use of agricultural chemicals etc. have complicated matters further which have in turn stimulated research on their biological control. Diaretiella rapae (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) is a common cosmopolitan parasitoid associated with various aphid species in all the major agro ecosystems.

D. rapae was originally described by Mcintosh in 1855 as Aphidius rapae. This species was brought under the genus Diaretiella by Stary (1961). Although of a very minute size, this parasitoid can be recognized by the following characters: antenna filiform, 13-17 segmented; wings hyaline, veins light brown; pterostigma triangular; fore wing with reduced wing venation to the extent that only a single large median cell is present; marginal cell distally open; r and RS of medium length, slightly curved, not reaching the wing margin; propodeum distinctly areolated with a narrow central areola medio-posteriorly; mesopleuron with a deep, finely crenulated transverse carina ("sternalus"); ovipositor sheath more or less straight or little curved upwards with obtuse apex and sparse hairs.

D. rapae, the only species known in this genus is commonly associated with aphids infesting cruciferous crops. The adult females lay eggs singly inside an aphid's body. Parasitized aphids can be easily identified by naked eye by their distinctive
appearance. After death mummified aphids consisting of the hardened exoskeleton turns golden yellow to golden brown and shiny. The wasp pupates within the aphid host and emerges from the mummy as an adult after cutting a circular smooth escape hole in the posterior-dorsal abdominal region.

Although it has been recorded from many parts of our country, viz., Assam (Rao et al. 1969); Delhi (Dey and Manikandan, 1996); Himachal Pradesh (Saha et al. 1982); Jammu and Kashmir (Bhagat and Ahmad, 1991; Takada and Rishi, 1980); Karnataka (Sethumadhavan and Dharmadhikari, 1969); Manipur (Subhrani et al. 2006); Meghalaya (Stary and Ghosh, 1978) and has been recognized as an important factor for the natural control of *L. erysimi* (Pike et al., 1999; Dogra et al., 2003; Dhiman, 2007; Akhtar et al., 2010) yet hardly any comprehensive work has been done on this parasitoid.

Considerable work has been done on the morphometrics of Braconidae (Tomic et al., 2005; Billah et al., 2008), but morphological variability of this important biological control agent, *D. rapae* has got little attention. Akhtar et al., (2011) redescribed *D. rapae* and presented a brief account of the morphological variability.

Considerable work has been done to assess the role of *D. rapae* in suppressing *L. erysimi* in mustard. Dhiman (2006) reported that *D. rapae* parasitize *L. erysimi* up to 68.69 per cent in field and 98.92 per cent in the laboratory. *D. rapae* was found to parasitize cabbage aphid, *Brevicoryne brassicae* L. up to 37.64 per cent in Egypt by Saleh et al. (2009). On the other hand, *D. rapae* females were found to be attracted by crucifer plants than by other types of plants (Sheehan & Shelton, 1989; Vaughn et al., 1996). Furthermore, *D. rapae* and *L. erysimi* prefer the same host plant possibly because both of them positively respond to the volatile compounds produced by the plants (Bundemberg, 1990).

Considerable work on its hosts, seasonal occurrence and parasitic potential have been carried out (Akhtar et al., 2010; Dhiman, 2007; Dogra et al., 2003; Pike et al., 1999) but a holistic approach involving emphasis on both ecological and taxonomic studies has not been applied to this cosmopolitan species. The original description is sketchy and morphological variations have not been quantified. Moreover hardly any work has been done to study inter and intra-plant distribution of *D. rapae* in mustard at field level.
Considering their biodiversity, abundance in diverse habitats and ecological importance, there is ample scope for its exploitation for management of aphid pests in various agro-ecosystems. Keeping in mind the above, the present study has been formulated with the following objectives.

1. Biosystematic studies on different populations of *D. rapae*

2. Study of distribution of *D. rapae vis-a-vis* its aphid host on mustard
BACKGROUND

All the pertinent literature on the biosystematics of parasitoid *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) vis-a-vis its aphid host has been reviewed under the following subheadings.

2.1 Biosystematics of *D. rapae*

2.1.1 Systematic history

2.1.1.1 World

The Hymenoptera is one of the largest orders of insects and frequently divided into two suborders, the Symplyta and the Apocrita. Nearly all parasitic hymenoptera are placed in the suborder Apocrita. Among the parasitoids are the two largest families, the Ichneumonidae and Braconidae. The family Braconidae is further subdivided into 34 subfamilies including Aphidiinae (Wharton, 1997). The present classification of Aphidiinae is the culmination of accumulated efforts over a century. They have been recognized as an insect group since the 17th century. The oldest published information on this group is found in Leuwenhoek’s Arcana Naturae (1695). The tenth edition of Systema Naturae (1758) by Linnaeus included *Ichneumon aphidum*, but unfortunately the type was lost so the true identity remained unknown.

*D. rapae* was originally described by McIntosh in 1855 as *Aphidius rapae*. Szepligeti (1904) in the 22nd volume of “Genera Insectorum” included it among known 14 genera and 170 species.

Genus *Diaeretiella* was erected by Stary (1960) with the following characters: head transverse, as wide as or wider than thorax at tegulae; antennae filiform, with variable number of segments (12 to 18); eye of medium size; mandibles bidentate. Notaulices developed on the fore part of mesoscutum. Propodeum distinctly areolated. Fore wing with pterostigma triangular; metacarp longer than width of pterostigma; radial vein developed, not longer than ⅔ of its possible length; otherwise venation effaced beyond basal cell towards the apex except cubital cell 2 and indicated part of cubital vein. Hind wing with complete basal cell. Abdomen of female lanceolate;
ovipositor sheaths and ovipositor straight or slightly curved upwards, sparsely haired and *Aphidius rapae* was transferred to this genus. Later on Stary (1966) published a review of Czechoslovakian Aphidiidae wherein he included synonymies, distribution, habitat, hosts, economic importance etc. of each aphidiid genus. He also described host parasitoid relationships, bionomics, ecology, parasitism, etc. Further in 1970, his publication “Biology of aphid parasites (Hymenoptera: Aphidiidae) with respect to integrated control” included 30 genera along with their bionomics, life cycle, phylogeny and distribution along with a key to genera and sub genera of the world. In another publication on “Aphid parasites (Hymenoptera: Aphidiidae) of the Mediterranean area” Stary, 1976 gave a critical synthesis of the characteristics and peculiarities of the Mediterranean fauna and discussed the potential of aphidiid parasitoids for biological control. In all of the above, *D. rapae* was recognized as a principal natural enemy in regulating the abundance of aphids infesting cruciferous plants.

Mackauer (1968) catalogued 32 genera, ten subgenera, 297 species and seven subspecies including *D. rapae* under subfamily Aphidiinae. *D. rapae* also finds mention in the first volume of “Catalogue of Hymenoptera of America north of Mexico” (Marsh, 1979).

Kavallieratos *et al.* (2001) presented the identification of genus and species with the observed parasitoids-aphid relationship on various host plants. Later during 2004 they reported 22 Aphidiinae genera and 115 species associated with 208 aphid taxa occurring on 422 plant taxa and 561 parasitoid-aphid associations. In 2005, they revised the genus *Praon* of southeastern Europe. In these work role of *D. rapae* was also highlighted.

Takada in 2002 discussed parasitoids of four principal pest aphids *viz.*, *Aphis gossypii, Myzus persicae, Macrosiphum euphorbiae* and *Aulacorthum solani* on greenhouse vegetable crops in Japan. *D. rapae* was confirmed along with other 12 species of Aphidiinae under 7 genera as parasitoids of the four pest aphids.
2.1.1.2 India

The first published information on aphid parasitoids in India dates back to 1912 when Viereck described a new species of Aphidiinae from Bangalore, South India.

Batra and Wadhi (1962) in a short account of some insect pests of economic importance, identified parasitoids emerging from parasitized aphids on cabbage plants as *D. rapae*.

Kundu *et al.*, (1966) recorded *D. rapae* (Curtis) as a parasite of mustard aphid, *L. erysimi* for the first time from India. Dharmadhikari and Ramaseshiah (1970) recorded *D. rapae* among the various natural enemies of aphids in India.

In 1983, Stary and Ghosh published a book titled “Aphid parasitoids of India and adjacent countries (Hymenoptera: Aphidiidae)” which reviewed nearly 70 species under 21 genera including *D. rapae*. The review also included information of the original description, type material, figures, hosts, biologies and distribution of taxa published up to 1981 and part of 1982. The host parasitoids index listed parasitoids records on 46 genera and about 100 species of aphids, irrespective of taxonomical group of aphids.


2.1.2 Morphometric studies

Although while redescribing *D. rapae*, Stary (1961) gave morphometric details of a few taxonomic characters like “… temple as wide as transverse eye-diameter; gena as wide as 1/3 to 1/7 of longitudinal eye-diameter; tentorio-ocular line 1/4 to 1/6 as long as intertentorial line; F1 and F2 of equal length, 2.5 times as long as wide; tergite I about 3 to 3.5 times longer than wide at spiracles…” but he did not quantify many important characters like: length of last flagellomere/length of
flagentomere I; length of last flagellomere/width of last flagellomere; tenterio-ocular line/inter tentorial line; length of median longitudinal carina on propodeum/length of dorsal carina on first tergum; width of central areola/width of mesopleuron; length of fore wing/maximum width or fore wing; width of stigma/length of stigma; length of ovipositor sheath/length of tergite I; length of ovipositor sheath/width of ovipositor sheath at base and number of lateral carinae on tergite I.

While working on morphological variability of several biotypes of *Ephedrus plagiator* (Nees, 1811), Tomnic *et al.* (2005), recomended the use of ratios in studies of numerical taxonomy as measures of shape following the principal laid by Hills (1978) and Dodson (1978). Although considerable work has been done on the morphometrics of Braconidae (Tomic *et al.*, 2005; Billah *et al.*, 2008), morphological variability in intraspecific population of *D. rapae* has got little attention. Akhtar *et al.* (2011) presented a brief account of morphological variability among four populations from India on the basis of ten character ratios, using numerical methods. His investigation indicated that the specimens reared from different localities showed clear distinction from other populations.

**2.1.3 Molecular studies**

Baker *et al.*, (2003) investigated the effects of colonization on *D. rapae* in Western Australia. When they compared with populations from the Old World, the results of a microsatellite analysis show that the insects have low allelic length and low allele frequency variation, revealing that those individuals experienced a significant founder effect. Marked genetic differentiation between populations was also revealed, which had potentially important implications for host utilization in *D. rapae* when introduced to a new geographical area. Low genetic variation and gene flow in a founder population could limit evolutionary potential in Australia, including the ability of a population to mount a response to newly introduced hosts, such as the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). Further they also mentioned that the actual importance of genetic diversity in the success of biological control agents was unclear.

Five polymorphic microsatellite markers were developed by Macdonald *et al.*, 2003 to investigate the spatial scales over which *D. rapae* moves within an
agricultural landscape. The number of alleles per locus ranged from 11 to 21. Cross-
species amplification indicated that those loci could be useful for some other members 
of subfamily Aphidiinae also.

Baer et al., (2004) investigated the phylogeography of *D. rapae*. To determine 
whether *D. rapae* populations collected from different aphid hosts have diverged into 
genetically independent lineages, they constructed a haplotype network based on 
sequence variation in mitochondrial DNA (mtDNA) by using single strand 
conformation polymorphism (SSCP) analysis to examine 2041 base pairs of mtDNA 
and to identify nucleotide sequences of 42 unique SSCP haplotypes. They found no 
association between mtDNA haplotypes and host species in either the ancestral range 
(Europe, Mediterranean region, Middle East, Asia) or part of the introduced range 
(western North America). Haplotypes likely to be ancestral were geographically 
widespread and found on both hosts, suggesting that the ability to use both hosts 
evolved prior to the diversification of the mtDNA. They concluded that ongoing gene 
flow appears to prevent the formation of host races.

Host-related fitness trade-offs in *D. rapae*, a generalist aphid parasitoid 
attacking more than 60 aphid species was investigated by Antolin et al., 2006). The 
study demonstrated that the wasps had higher productivity and survival when 
attacking 'home' hosts than 'alternate' hosts, and trade-offs were found by quantitative 
genetic analyses to be genetically determined. Although the fitness differences 
described in the study could be strong enough to create populations adapted to 
different hosts, but it appeared that gene flow was sufficient to prevent formation of 
separate lineages on two hosts. Thus they concluded that rather than being a generalist 
with a broad host range, *D. rapae* is a serial specialist, attacking particular hosts 
according to availability in different seasons or in different geographical areas.

2.2 Distribution and seasonal occurrence of *D. rapae* vis-a-vis *L. erysimi*

2.2.1 Host plant: Rapeseed-Mustard and Host aphid: *L. erysimi* (Homoptera: 
Aphididae)
Laboratory studies conducted on adults of *D. rapae* derived from mummies of *L. erysimi* on *Brassica oleracea* Tripathi and Tripathi (1991) reported pivotal role of temperature on the survival of *D. Rapae*.

Shukla *et al.*, (1992) assessed the effect of different food plants viz., cabbage, mustard and radish on the functional response of *D. rapae*. They found that the female parasitoid located plant material, stayed longer on the leaf surface, contacted more hosts and parasitised a greater number of hosts, particularly at increased host densities, on cabbage than on either of the other plant species. Further, Shukla and Tripathi (1993) assessed the effect of different food plants on the offspring sex ratio of *D. rapae* on *L. erysimi* and found *Brassica oleracea* as the most suitable food plant for mass rearing of *D. rapae* in the Laboratory. Again, Shukla *et al.*, (1997) studied the numerical aspects of interactions between *D. rapae* and its host aphid *L. erysimi* bred on three different brassicaceous plants at different parasitoid densities. They found that the killing power (k-value) of the parasitoid increased significantly with an increase of parasitoid density on all the plants but the rate of multiplication, expressed as parasitoids egressed/female parasitoids decreased with an increasing parasitoid number and also in the sequence of *Brassica oleracea* cv. Pride of India, *B. campestris* cv. Varuna and *Raphanus sativus* cv. Japani white. Thus the fact that more number of parasitoids lowered the rate of multiplication showed the existence of mutual interference among *D. rapae*. Further Shukla (2001) investigated the effect of three food plants of *L. erysimi* on the area of discovery and killing capacity *D. rapae*. He reported that with increase in parasitoid density, the area of discovery/28.3 cm² significantly decreases, this decrease being least in aphids bred on *B. oleracea*, followed by *B. campestris* and *R. sativus* bred aphids. He also found that the area of discovery/28.3 cm² and k-value (killing capacity of the parasitoid) of a female parasitoid increases up to 50 hosts and thereafter, decreased.


### 2.2.2 Parasitoid *D. rapae*

#### 2.2.2.1 World
The effect of temperature on longevity, reproduction, and development of the asparagus aphid and *D. Rapae* was studied in Japan by Hayakawa et al., (1990).

Laboratory and field experiments to determine whether cabbage aphids separated from colonies were subject to higher rates of successful parasitoid attack, or whether parasitized aphids were more likely to become separated from colonies because of increased rates of movement over the plant revealed that parasitism levels of 'scattered' aphids on potted kale plants exposed for 3 days in a kale field were 2.3-3.2 times higher than parasitism levels for aphids in colonies on identical potted plants at the same location for the same period (Lopez et al., 1990). Thus concluding that higher levels of parasitism in 'scattered' aphids seen on field plants were probably the result of the combined effects of higher levels of successful parasitoid oviposition in aphids already outside of colonies and higher rates of movement of aphids away from colonies after colonies are attacked by parasitoids.

Titayavan and Altieri (1990) reported synomone-mediated interactions between the *D. rapae* and *B. brassicae* under field conditions. Their results suggested the existence of a synomone-mediated interaction between the species involved, indicating potential avenues to enhance field parasitization rates through manipulation of the chemical environment of cole cropping systems.

A study to evaluate the tritrophic interactions between resistant and susceptible barley, triticale and oats, *Diuraphis noxia* and *D. rapae*, and their efficacy in reducing the damage to those food plants indicated that there was a significant decrease of *D. noxia* in the plant entries tested when a single parasitoid was allowed to oviposit for 24 h (Reed et al., 1991). They further in 1992 developed cohort life tables in the laboratory for *D. rapae* and *Aphidius matricariae* on *D. noxia*. Their study revealed that female *D. rapae* began to oviposit at a younger age (13 days) than *A. matricariae* (14 days). Age-specific fecundity for *D. rapae* peaked at approximately 18 eggs/female at 15 days. Overall sex ratio of progeny was strongly (66.9%) female biased for *D. rapae* and net reproductive rate was 58.6 female offsprings/female. Mean generation time for *D. rapae* was approximately 2.5 days shorter than for *A. matricariae*. The intrinsic rate of increase was reported greater for *D. rapae* (0.263) than for *A. matricariae* (0.202).
Bernal, and Gonzalez (1995) undertook studies to assess the effect of temperature on development, and to estimate the thermal requirements (\( t \) and \( K \)) and upper developmental thresholds of three developmental periods (egg-pupa, pupa-adult and egg-adult) in *D. rapae* on host *Diuraphis noxia*. In general, they showed that the developmental times and rates for the three developmental periods in *D. rapae* were influenced by the temperature to which they were exposed. They also found that the estimated \( t \) and \( K \) values were 2.47°C and 198.99 degree-days, resp., for the egg-pupa period; 3.89°C and 106.45 degree-days, resp., for the pupa-adult period; and 3.56°C and 292.29 degree-days, resp., for the egg-adult period.

The response of mated *D. rapae* females to odours from wheat, cabbages and plant-host complexes using a 4-choice olfactometer was investigated by Reed et al., (1995). They found that the response of *D. rapae* to the cabbage-*B. brassicae* complex and to *B. brassicae* alone was significantly greater than to the wheat-*D. noxia* complex and *D. noxia* alone, suggesting an innate odour preference for crucifer-feeding aphids.

Vaughn et al., (1996) investigated behavioral and physiological responses of *D. rapae* to semiochemicals and recorded that males and females responded similarly to (Z)-3-hexen-1-ol, a component of green-leaf (Brussels sprout or grass) volatiles, in both electroantennogram and flight tunnel assays. Females responded similarly regardless of prior oviposition experience or population origin. Results suggested that mating history and aphid presence did not increase responsiveness toward these semiochemicals.

Reproduction behaviour of *D. rapae* on Russian wheat aphid hosts at different temperatures was studied in the laboratory. Bernal and Gonzalez (1997) found pre-imaginal survivorship similar among temperatures, while \( R_o \) and \( T_c \) decreased with temperature, and \( r_m \) increased with temperature. The offspring sex ratio (proportion females) was lowest at 26.7 degrees C, and similar between 10.0 and 21.1 degrees C. In addition, the offspring sex ratio significantly declined with the age of the female parent.

Brewer et al., (1998) explored the interaction of regulating agents of Russian wheat aphid (RWA; *Diuraphis noxia*) that have been introduced (parasitoids) and
other factors (resistant barley STARS-9301B) to determine if parasitism of RWA by *Aphelinus albipodus* and *Diaeretiella rapae* might be affected by susceptible (cv. Morex) and resistant plant types. Results showed that *D. rapae* was more effective in parasitizing relatively high densities of RWA within curled leaves of Morex than relatively low densities of aphids on uncurled leaves of STARS-9301B. Their findings suggest that host plant resistance and biological control are compatible, but not synergistic.

Pike *et al.*, (1999) studied host range and habitats of *D. rapae* in Washington State and found nineteen species of aphids positively linked with *D. rapae* among which six species, viz., *Acyrthosiphon lactucae*, *Phorodon humuli*, *Dysaphis plantaginea*, *Brachycaudus tragopogonis*, *Uroleucon ivae* and *Braggia* spp. were reported as hosts of *D. rapae* for the first time. *D. rapae* was shown to switch or alternate successfully between hosts, which suggested that certain host habitats might be used in conjunction with farmlands to enhance host opportunities for the parasitoid and improve its population stability. They also concluded that, *B. asparagi* and *D. noxia*, were less problematic in Washington, in part at least because of host switching and acceptance by *D. rapae*.

Bradburne and Mithen (2000) described the differential attraction of *D. rapae* parasitizing *Brevicoryne brassicae* to two near-isogenic lines of *Brassica oleracea* which differ in a gene which alters the chemical structure of the isothiocyanates which are emitted following tissue damage. Further, they demonstrated that, by enhancing the production of but-3-enyl isothiocyanate in *B. oleracea* and *B. napus* (oilseed rape), the attraction of *D. rapae* to these plants under standard field conditions can be enhanced.

Qayyum (2001) assessed the effect of host age on two closely related parasitoid species *D. rapae* and *Aphidius colemani*. In his experiment *A. colemani* was recorded to produce significantly more mummies than *D. rapae* in 2- and 4-day-old aphid (*Myzus persicae*), whereas *D. rapae* produced more mummies in 6- and 8-day-old aphids. Both the parasitoid species were found capable of successfully ovipositing in any developmental stage of *M. persicae*. The results suggested that better parasitism should be expected at the beginning of the aphid infestation season
from *A. colemani*, because of the fact that early stages were more numerous than older hosts while at the end of aphid infestation season, better parasitization from *D. rapae* should be expected when the older hosts were more numerous than the young ones.

Patch and prey utilization behaviour of *Aphelinus albipodus* and *D. rapae* was studied on Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae). Lester and Holtzer (2002) observed individual female parasitoids for 1 h after arriving on a wheat plant with varying *D. noxia* densities and recorded that the total amount of time spent on the leaf and the number of occasions a parasitoid left the leaf were dependent on aphid densities for *D. rapae*. After 1 h on the wheat plants, single *D. rapae* females produced up to 31 progeny from 40 aphids, while single *A. albipodus* produced a maximum of six progeny. The mean oviposition time for *A. albipodus* was 119 s compared to 1 or 2 s for *D. rapae*.

Jankowska and Wiech (2003) recorded occurrence of *D. rapae* in *B. brassicae* colonies on different cruciferous crops. Blande *et al.*, (2004) assessed foraging behavior of *D. rapae* with *L. erysimi* and *M. persicae* using a series of attack rate and success bioassays, with turnip, *Brassica rapa* var *rapifera*, as the host plant. They discovered that attack rate of *D. rapae* was significantly greater on *L. erysimi* than on *M. persicae* when aphids were feeding on turnip leaf discs in Petri dishes, irrespective of the aphid species upon which the parasitoids were originally reared. However, the relative success of *D. rapae* on these two aphid species, in terms of the percentage of attacks resulting in a successful adult parasitoid, was not significantly different.

Leaf epicuticular wax effected the movement, foraging behavior, and attack efficacy of *D. rapae*. The parasitoid foraged more slowly on a variety with a heavier wax bloom, groomed more often and for longer periods of time, fell from the leaves more often, took longer to find colonies of aphids, and attacked them at a lower rate than wasps foraging on a variety with a lighter wax bloom Gentry and Barbosa (2006).

Using a Y-tube olfactometer, Girling *et al.*, (2006) investigated responses of *D. rapae* to volatiles from *Arabidopsis thaliana Columbia* (Brassicaceae) induced by *Myzus persicae*. In dual-choice experiments, female *D. rapae* given oviposition experience on *A. thaliana* infested with *M. persicae* were significantly attracted to
volatiles from *A. thaliana* infested with *M. persicae* over volatiles from undamaged *A. thaliana*. They concluded that an interaction between the plant and the aphid induces *A. thaliana* to produce volatiles, which *D. rapae* can learn and respond to.

Similar studies by Agbogba and Powell (2007) on responses of *D. rapae* to odors from cabbage plants infested with *M. persicae* (Sulzer) (Homoptera: Aphididae), in both the presence and absence of a lepidopteran caterpillar, *Plutella xylostella* L. (Lepidoptera: Plutellidae) showed that female parasitoids chose aphid-infested plants over uninfested plants but did not distinguish between caterpillar-infested and uninfested plants. When given a choice between odors from an aphid-infested plant and those from a plant infested with diamondback moth larvae, they significantly chose the former. Their study hypothesized that the aphid and the caterpillar induce different changes in the volatile profile of cabbage plants and *D. rapae* females readily distinguish between the two.

Bayhan *et al.*, (2007) studied development time and parasitization rate of *D. rapae* on *B. brassicae* feeding on different *Brassica* cultivars in the laboratory at 20°C and demonstrated that the parasitism rate could be influenced by the plant quality, probably due to the nutritional status of the aphids or to toxic compounds ingested through the plant. Cabbage, cauliflower and broccoli were found to be suitable plants for the parasitoid, considering the development time of pre-adults, and the parasitization rate of *D. rapae* on *B. brassicae*.

In a study on the host searching, handling and oviposition behaviour of *D. rapae* in relation to host age Kant *et al.*, 2008 found that the parasitoid spent 61% of her foraging time searching for hosts. Host handling time of the parasitoid decreased with increased number of host encounters. The females were more successful in finding older hosts (7 days old) and spent more time (94.9±20.5s/encounter) and stung them more (9.9±1.4/encounter) than the younger hosts. They preferred to sting the abdomen rather than the thorax, head or legs of the host. The average number of eggs laid per host was highest (1.4±0.2 eggs) in 7-day-old hosts. When attacking 7-day-old hosts, they gained 42% success in ovipositing the host compared to 10, 18 and 30% success in 1-, 3- and 5- day old hosts, respectively. They concluded that *D. rapae* may have adaptive preference for larger hosts and mass
production could be more efficient by using 7-day-old aphids. Kant and Sandanayaka (2009) studied diel variation in emergence, mating and oviposition of *D. rapae* in the laboratory to understand the biology and behaviour of the parasitoid. They found greatest emergence during the early photophase. The parasitoids that emerged during the scotophase did not mate until the following photophase. Their research suggested that light triggers parasitoid activity and that the parasitoids lose their reproductive fitness if they emerge in the scotophase.

Pope *et al.*, (2008) compared innate responses of the aphid parasitoid *D. rapae* to alkenyl glucosinolate derived isothiocyanates, nitriles, and epithionitriles. Electroantennogram responses indicated peripheral odor perception in *D. rapae* females to all 3-butenylglucosinolate hydrolysis products tested. In contrast, of the 2-propenylglucosinolate hydrolysis products tested, only the isothiocyanate elicited significant responses. Despite showing peripheral olfactory detection of a range of 3-butenyl glucosinolate hydrolysis products, native females oriented only to the isothiocyanate. Similarly, parasitoids showed orientation to 3-isothiocyanatoprop-1-ene, but not to the corresponding nitrile or epithionitrile.

A study conducted by Saleh (2008) in Sharkia Governorate, Egypt showed that, the longevity of *D. rapae* parasitizing different aphid species was affected by temperature and food but the sex ratio were not affected by host species. Behavioural study of this parasitoid at varying host densities showed a decrease of host-searching and first sting times with increasing of host density but number of stings and number of mummies increased with increase of host density.

Kissen *et al.*, (2009) used *Arabidopsis thaliana* as an experimental model plant to investigate a tritrophic interaction between the plant, a specialist aphid herbivore, *B. brassicae*, and its natural enemy, the parasitoid *D. rapae*. The *A. thaliana* ecotype Col-5 was transformed with a functional 2-oxoglutarate dependent dioxygenase (BniGSL-ALK). This transformation resulted in a change in the glucosinolate hydrolysis profile of the plant. Performance of *B. brassicae* was affected negatively by transforming Col-5 with BniGSL-ALK in terms of mean relative growth rates. In a series of behavioral bioassays, naive *D. rapae* females were able to discriminate between *B. brassicae* infested and uninfested Col-5 plants.
transformed with BniGSL-ALK, with parasitoids showing a preference for *B. brassicae* infested plants. In contrast, naive *D. rapae* females were unable to discriminate between aphid infested and uninfested Col-5 plants.

### 2.2.2.2 India

Vekaria and Patel (2000) explored the possibilities of delaying emergence of *D. rapae* through refrigeration of mummified mustard aphid at 8-10°C. Eclosion of mummified aphids was 100% after 6 days of refrigeration and decreased thereafter. They found 40% adult eclosion even after 120 days of refrigeration.

Dhiman and Singh (2002) studied feeding and migratory behaviour of *D. rapae*. Later in 2005, Dhiman examined the feeding potential of *D. rapae* on *L. Erysimi*. He reported that the third instar larva voraciously fed on the aphid tissue and the abdomen of the aphid was cleaned out first and then the thorax and head. Food material from the legs and antennae were devoured by suction process. Final and fourth instar larvae scraped the integument by mandibular sclerite and consumed everything within the aphid, except for trachea. Lastly, fourth instar larvae made a hole in the venter of the aphid and glued it to the substratum and then pupated inside the aphid by making thin silken puparium.

### 2.2.3 Role as a parasitoid

Bernal *et al.*, (1994) conducted functional-response experiments involving *D. rapae* and its host *Diuraphis noxia* with host densities 10, 20, 40, 80, and 160 hosts/arena per day. In their experiment, the offspring sex ratio did not appear to vary significantly with a host density of up to 80 hosts/ female per day although the proportion of female offspring increased when female parasitoids were offered 160 hosts/ day. The maximum attack rate per 24-h period was 59.5 mummies.

Laboratory studies were conducted to determine the suitability of 16 aphid species as hosts for *D. rapae*, with a view to identify alternative hosts for the purpose of field releases Elliott *et al.*, (1994). Of the 14 species tested, they found females of *D. rapae* parasitized and developed to adulthood in only seven species, *viz.*, *Diuraphis noxia, Rhopalosiphum maidis, Schizaphis graminum, R. padi, Lipaphis erysimi, Aphis gossypii* and *B. brassicae*. In an another study Elliott *et al.*, (1994) found a greater
percentage of parasitized *B. brassicae* (94%), compared to other species and further the dry weight of adults eclosing from *B. brassicae* was greater than for other species.

Qayyum (2000) compared some fitness parameters of *D. rapae* and *A. colemani* reared on *M. persicae* and reported *D. rapae* as superior species in terms of parasitoid fitness for biological control, because of its shorter generation time compared to *A. colemani*.

Parasitism as high as 89.7% due to *D. rapae* on *L. erysimi* and *B. brassicae* at flowering and pod developing stage of *Brassica napus* in Mato Grosso do Sul, Brazil was reported by Mussury and Fernandes (2002).

Studies during 1992 to 1999 evaluated the role of Savoy cabbage and turnip as banker plants in the enhancement of the action of *D. rapae* against *Brevicoryne brassicae* in cauliflower in Switzerland. *D. rapae* was seen to dominate the aphid parasitoid community with >90% parasitism (Freuler et al., 2003).

Cage experiments with *D. rapae* to control *B. Brassica* recorded that at the ratio of 1.2:1 (six female wasps: five aphid adults), and three releases on day 1, 3 and 6, the percent parasitism of the offspring on day 20 was 88.9%, and the aphids were successfully controlled by the second generation of the released parasitoids (Zhang and Hassan, 2003). Further when cabbage plants that included both mummies and young aphids were used in a broccoli field, with a dose of about 2 mummies/m², resulted in higher percentage of the broccoli plants with mummies in the treated plot compared with the control plot. Seven weeks after the release of the parasitoid, the percentage of the broccoli plants with mummies was 93.3% in the treated plot and 56.7% in the control plot; the percent parasitism of the aphids were 6.7 and 1.4% in the treated and control plots, respectively. Although their experiment indicated that one release of the cabbage plants with mummies enhanced the spread of the parasitoid *D. rapae* and increased parasitism of the aphids in the field, more releases of the parasitoid were needed to control the aphid effectively.

Kavallieratos *et al.*, (2004) carried out a field experiments in a citrus orchard in Greece for two years (2000 and 2001) to examine the role of aphidophagous insects in the population reduction of aphids infesting citrus. The aphid species found were:
Toxoptera aurantii, Aphis gossypii and M. persicae. Among these species T. aurantii was by far the most abundant. The aphidiine parasitoids recorded from T. aurantii in decreasing order of occurrence were A. colemani, A. matricariae, D. rapae, Praon volucre and Ephedrus persicae while in the case of A. gossypii, the aphidiine parasitoids emerged in decreasing order of occurrence were Binodoxys angelicae, A. colemani and D. rapae. In contrast, M. persicae was not found to be parasitized. The parasitization of T. aurantii was significantly higher than that of A. gossypii.

Desneux et al., (2005) studied the impact of D. rapae on populations of M. persicae when parasitoids were introduced on deltamethrin-treated plants at increasing intervals after treatment. They found that both deltamethrin and D. rapae exerted additive effect on aphid mortality. Their work suggested that D. rapae could limit populations of M. persicae, even after pyrethroid treatment, as the presence of deltamethrin residues had little impact on the parasitoid.

Studies on the functional response and mutual interference of D. rapae attacking B. brassicae revealed that per capita parasitism decreased significantly from 80.80 (67.33%) to 11.85 (9.88%) as parasitoid densities increased from 1 to 8 females (Fathipour et al., 2006). Consequently, the per capita searching efficiency decreased significantly as parasitoid densities increased. The rate of parasitism increased as the host density increased. Therefore, they concluded that different host-parasitoid ratios could affect the efficacy of D. rapae.

Blande et al., (2007) reported that D. rapae, attacked L. erysimi, a specialist feeding aphid of the Brassicaceae, at a greater rate than the generalist-feeding aphid, M. persicae. They investigated the orientation behaviour of D. rapae to the volatile chemicals produced when these two aphid species feed on turnip (Brassica rapa var rapifera). Further they also assessed parasitoid orientation behaviour in response to laboratory-formulated isothiocyanates indicated significant orientation toward 3-butenyl isothiocyanate.

Ponti et al., (2007) assessed the effects of crop diversification levels and fertilization regimes on abundance of Brevicoryne brassicae (L.) and its parasitization by D. rapae in broccoli and reported that, aphid pressure decreased and natural enemies of cabbage aphid were enhanced in intercropping treatments, but this varied
with the intercropped plant and season (summer vs. autumn). In compost-fertilized broccoli systems, seasonal parasitization rates of *B. brassicae* by *D. rapae* increased along with the expected lower aphid pressure compared with synthetically fertilized plants.

A demographic and modeling approach was used to determine the suitability of two hosts, *B. brassicae* and *M. persicae* for *D. rapae*. An examination of key life history parameters indicated that *M. persicae* was the better host of the two as net replacement rate, birth rate, intrinsic, and finite rates of increase were all higher in *D. rapae* that developed in *M. persicae* than in *B. brassicae*. Although generation time was slightly shorter for *D. rapae* reared on *B. brassicae* than on *M. persicae*, a population model based on demographic data indicated that *D. rapae* populations would grow much faster when developing on *M. persicae* compared to *B. brassicae*. Thus Stark and Acheampong (2007) concluded *D. rapae* to be a more effective biological control agent for *M. persicae* than for *B. brassicae*.

Saleh *et al.*, (2009) conducted a study at Kafer Sakr district, Sharkia Governorate, Egypt for the four seasons during 2005-09 recorded rate of parasitism by *D. rapae* (M’ Intosh) on the cabbage aphid, *B. brassicae* as 29.87, 36.44, 25.74 and 37.64 per cent respectively. Respective total means of percentage adult emergence were 82.74, 80.09, 81.71 and 81.81 per cent. Further they found that *D. rapae* abundance was negatively correlated with temperature in the four seasons and *D. rapae* had significant negative correlations with the host. Parasitoid density in relation to host density influenced percentage of parasitism. Highest percentage parasitism was 91.40 per cent at 16 *D. rapae* females per cage while the minimum was 55.6 per cent at one female per cage.

In India, Dhiman (2006) reported variable percentage of parasitisation by *D. rapae* according to the host species and climatic conditions. At Saharanpur, U.P it varied from 46.57 to 68.69% in the field and 91.13 to 98.92% in the laboratory. He also reported multiple parasitism or superparasitism although, only one parasitized larva developed within a single host.

Raj and Sharma (1993) reported prevalence of *D. rapae* on aphid complex infesting rapeseed at Palampur, Himachal Pradesh with percentage parasitism
varying from 2.40 to 36.89 and 8.65 to 11.02 in *L. erysimi* and *B. brassicae*, respectively and also preference of *D. rapae* preferred for *L. erysimi* over *B. brassicae* under field conditions.

### 2.2.4 Distribution

Spatial distribution of an animal species is essential in ecological research for a better understanding of population dynamics. It is one of the most characteristic ecological properties of species, unlike rates of growth and reproduction, which often vary more between generations within a species than they do between species. Spatial distribution yields characteristic parameters that segregate species. These parameters are the population expression of the individual behavior defined by the ecologist and observed by the naturalist. No field sampling is viable without understanding spatial distribution. Some insect species reproduce so rapidly that population density can change greatly during the course of a field experiment as in the case of aphids (Hassell and May, 1974 and Anderson, 1974).

Kavallieratos *et al.*, (2005) conducted field studies in western Greece during 1996 and 1997 growing seasons, in order to assess the seasonal occurrence and the spatial distribution of *A. colemani, Aphidius matricariae, D. rapae, Praon staryi* and *P. volucre*, all parasitoids of *M. persicae* (Sulzer) on tobacco. They collected tobacco leaf samples from the upper and lower half of plants. They found that the density of *M. persicae* was higher on the leaves collected from the upper part of the plants than on those from the lower part, but without significant difference. In contrast, the numbers of mummified *M. persicae* individuals were significantly higher on leaves collected from the lower part of the plants than on those from the upper part during both years.

The distribution of various species of *L. erysimi* on mustard have been studied extensively by Paras Nath and Mishra, 1986; Ramkishore and Phadke, 1988; Shukla, 1990; Rajenderan and Phadke, 1992 and Kuo MeiHwa, 1993. In all the studies, the population of the aphids was found to be aggregated and conformed to a negative binomial distribution. Dhiman (2007) observed maximum mummies on the ventral side of the leaves and inflorescence.
2.2.5 Seasonal occurrence

2.2.5.1 World

Boyd and Lentz (1994) studied seasonal incidence of aphids and aphid parasitoid *D. rapae* on rapeseed in Tennessee and recorded the highest *D. rapae* density as 883 wasps/50 sweeps.

In Poland, Gabrys *et al.*, (1998) investigated degree of natural reduction of *B. brassicae* population by *D. rapae* on different Brassicaceae crops in relation to their growing period and the cabbage aphid infestation period. In their experiment, the first parasitised aphids were found approx. 2 weeks after the first aphid colonies appeared and this coincided with maximum aphid number on any of the crops. Although initially, the percentage of parasitisation was very low (<5%), the maximum parasitisation (<35%) occurred 2 weeks after initial parasitisation and coincided with the decline of aphid population due to migration.

Geiger *et al.*, (2005) investigated the population dynamics of *Brevicoryne brassicae* and *D. rapae* in brussels sprout fields and adjoining flower plots in winter. Their findings suggest that flower species under investigation did not function as sources of *B. brassicae* and brussels sprout plants that were not harvested may harbour *D. rapae* populations along with *B. brassicae* as a source of infestation.

Saleh *et al.*, (2006) assessed the role of *A. colemani* and *D. rapae* as parasitoids on the common reed aphid, *Hyalopterus pruni* in Egypt and found that the correlation coefficient of temperature was significant with *A. colemani* and insignificant with *D. rapae* population density, while that of relative humidity was insignificant on both parasitoids.

2.2.5.2 India

Desh Raj (1998) assessed the efficiency of *D. rapae* on aphid complex infesting rapeseed in mid hill zone of Himachal Pradesh. In the field under nylon netting, he recorded maximum parasitisation of 21.05, 23.16 and 6.53 per cent during 1992-93; and 62.71, 55.67 and 17.18 per cent during 1993-94, on *L. erysimi*, *B. brassicae* and *M. persicae*, respectively. During both years, peak parasitisation by *D.
rapae was observed in the first fortnight of March. He recorded parasitoid activity from the first week of February to the first week of April when mean maximum and minimum temperatures varied from 14.3 to 25.08 degrees C and 5.1 to 14.3 degrees C, respectively.

Heavy infestation of L. erysimi, on Indian mustard cv. Varuna, during the first week of January 1991, was observed at Akola, Maharashtra and the percentage of mummified aphids varied between 20 and 91.62 per cent, with a mean of 66.68 per cent (Men and Kandalkar, 2001).

In an experiment conducted in Palampur, Himachal Pradesh Dogra et al., (2003) recorded maximum parasitization (51.07 per cent) of L. erysimi by D. rapae during the second week of March but they did not find any significant impact of temperature, relative humidity and rainfall on the populations of L. erysimi and D. rapae.

Dhiman (2007) studied the population dynamics of D. rapae on mustard aphid, L. erysimi. He reported that the population density of mummified aphids depended upon the density of adult parasitoids, host aphids and climatic factors (temperature, relative humidity, rain, wind velocity and light intensity etc.) with maximum population density of both adult and mummies in April-May and minimum during January-February and October.

Akhtar et al., (2010) evaluated the occurrence of D. rapae parasitizing L. erysimi in Mustard variety Pusa bold (Brassica juncea) and reported 75.46 per cent and 68.96 per cent successful parasitism during 2006-07 and 2007-08 respectively. Further average per cent relative humidity showed a significant negative correlation with both aphid (r=-0.52) and parasitoid (r=-0.59) whereas day (r=0.65) and the night temperature (r=0.61) had significant positive correlations with the parasitoid population only.

2.2.6 Hyperparasitism

Pachyneuron aphidis (Bouche) was reported as a hyperparasitoid of D. rapae by Pandey et al., 1985. Field studies by Freuler et al., (2003) in Switzerland from 1992 to 1999 revealed that D. rapae activity was hindered from the beginning of July
by hyperparasitoids such as *Alloxysta* spp., *Asaphes suspensus* and *P. aphidis* by which it was rapidly overrun. *Opius, Dendrocerus, Pachyneuron aphidis, Charips* and *Asaphes* spp. were reported as hyperparasitoids of *D. rapae* by Dhiman (2006). Saleh *et al.*, (2009) conducted a study at Kafer Sakr district, Sharkia Governorate, Egypt for the four seasons during 2005-09 and reported two hyperparasitoid species; *Pachyneuron* spp. and *Alloxysta* spp. on *D. rapae* with average per cent hyperparasitism of 4.84, 3.53, 5.68 and 3.40 per cent in the four seasons, respectively.
MATERIAL AND METHODS

3.1 Biosystematic studies on *Diaeretiella rapae*

3.1.1 Sources of biological materials

Eleven samples of *Diaeretiella rapae* populations were collected from nine different places of India (Table. 4.1). Six populations parasitizing *L. erysimi* on mustard were collected from Almora (23°36'06"N, 79°39'16"E; elevation 1456 m), Delhi (28°04'01"N, 77°09'06"E; elevation 223 m), Dehradun (30°19'17"N, 78°01'55"E; elevation 652 m), Indore (22°43'31"N, 75°51'56"E; elevation 511 m), Nainital (29°23'52"N, 79°26'08"E; elevation 2031 m) and Pantnagar (29°01'20"N, 79°29'15"E; elevation 234 m). Five populations parasitizing *Lipaphis erysimi* on cabbage were also collected from Aligarh (27°54'01"N, 78°04'58"E; elevation 191 m), Almora (23°36'06"N, 79°39'16"E; elevation 1456 m), Barapani (25°43'11"N, 91°58'43"E; elevation 906 m), Delhi (28°04'01"N, 77°09'06"E; elevation 223 m) and Pantnagar (29°01'20"N, 79°29'15"E; elevation 234 m). Collections were made within a week time period in order to maintain homogeneity of samples. Due care was taken to label these samples with data like host name, date of collection, locality and details of collector before suitably storing.

3.1.2 Preparation and Preservation

Adult parasitoids were preserved in 70% ethyl alcohol and later identified to the species using essential diagnostic characters under Leica MZ12 stereo zoom microscope with suitable illumination. Specimens in intact conditions suitable for morphometric analysis were selected and dry mounted on rectangular paper tags using a fine brush and needle. Each specimen was labelled with details of host, date of collection, locality and collector’s name and placed in insect boxes for further studies.

3.1.3 Selection of samples

These dry mounted specimens were individually examined under Leica WILD HEERBRUGG compound stereo zoom microscope at magnification of 40x to 100x to confirm uniformity and suitability for morphometrics. The physical condition like cleanliness, completeness and amenability for measurement were the criteria utilized
here for selection of specimens. Such good female specimens of nearly uniform and stable conditions with least variability were randomly selected and individually numbered before undertaking morphometric analyses.

3.1.4 Selection of characters

The available taxonomic literature on the descriptions or redescriptions of *D. rapae* and its diagnostics at different hierarchical levels were consulted for selection of characters. Studies by M’Intosh (1855), Mukerji and Chatterjee (1950), Stary (1961) and Akthar *et al.*, (2011) on the taxonomy and morphometrics of *D. rapae* were taken into consideration. Literature indicates that propodeal and fore wing characters are of extreme importance in identification of *D. rapae*, and hence all characters available on propodeum and fore wing were taken into consideration along with addition of some new characters (Table 4.2). Forty quantitative characters were measured in 167 specimens from eleven populations of *D. rapae* which were crucial in defining *D. rapae* at all its taxonomic levels (Table 4.2 and Fig. 4.1-4.8). The quantitative characters were of two kinds: meristic and ratios (Table 4.2). The use of ratios in studies of numerical taxonomy as measures of shape was recommended by Hills (1978), Dodson (1978) and Tomnic *et al.* (2005). We used logarithmic transformations of our data performed on the log(x+1) of both meristic and ratio characters (Hills 1978). All the 12 quantitative characters with descriptive statistics parameters and summary statistics of logarithmic transformed data have been listed in Table 4.3.

3.1.5 Study and measurement of characters

While studying the specimens under microscope, care was taken to ensure that each specimen are always in a level plane or set uniformly every time at the same angle. While doing so, the precautions to be taken at the time of microscopic examinations of taxonomic characters by different workers were kept in mind. All the measurements were taken under Leica WILD HEERBRUGG compound stereo zoom microscope at magnification of 130x except, number of lateral carinae on tergite I, which was counted at magnification of 320x fitted with ocular micrometer. The measurements in terms of ocular values thus converted into mm values using a stage micrometer. Such values were tabulated and then subjected to further analyses.
3.1.6 Data analysis

The measurement of taxonomic characters of *D. rapae* was tabulated separately for different populations. Morphometric analyses were performed using MS Windows (version 2007) operated MS Excel (version 2007) and Statistical Analysis System software version 9.2 (SAS Institute Inc., 2009). The main objective of the statistical analysis was to know the significance or otherwise of the variation exhibited by a character, between the individuals of a sample population and also between the various populations.

i. Coefficient of Variation (CV)

To evaluate the stability and consistency of a character and to assess the utility of a character for its diagnostic value, the coefficient of variation analyses were done for each character.

ii. Principal Component Analysis (PCA)

This statistical procedure was used to reduce the number of variables by projecting the original measured variables into a new set of variables that has some inherent statistical properties. This analysis is thus used to investigate morphological variations and thereby exploring differences among the groups by effectively reducing the dimension of the problem. The total number of principal components that could be generated is the total number of measurements considered. Since the number of measurement is very high, using the principal component analysis, it is possible to select first few principal components, which have large variations and could explain the differences in the populations.

Principal component analysis was performed on the correlation matrix for the 40 variables (2 meristic and 38 ratio characters) to determine the effects of size and shape on the distribution of scores along the first four principal component axes (Sokal and Rohlf, 1995) and observe their distribution without constraints of prior assignment to particular populations. Thereafter cluster analysis was performed following the AVERAGE method and a hierarchical cluster was constructed.
3.2 Study of distribution of *D. rapae* vis-a-vis its aphid host in mustard

3.2.1 Experimental site

The study was conducted at the research farm of Indian Agricultural Research Institute, New Delhi, India (28°4'N, 77°09'E and 223.06m above mean sea level) during two consecutive *rabi* seasons viz., 2009-10 and 2010-11. *Brassica juncea* variety Pusa bold was sown in 5 plots sized 8m×10m. Sowing was done on 17th November and 22nd November during 2009 and 2010 respectively. All the recommended agronomic practices were followed to raise a good crop. No insecticidal spray was given to avoid mortality of natural enemies.

3.2.2 Sampling

Sampling during the previous years had indicated a heavy parasitisation of *L. erysimi*. Sampling was from the first appearance of aphids on mustard plants which was 17th January and 23rd January during 2010 and 2011 respectively and continued till the harvest of the crop on 21st March and 27th March during 2010 and 2011 respectively. Sampling was done at weekly intervals and at each sampling five plants were chosen randomly from each plot i.e. samples were collected from a total of 25 plants. Each sampling unit comprised of three subunits, viz., terminal 10cm twig, one leaf each from the middle and lower region of the plant.

3.2.3 Processing and preservation of samples

Each plant part was enclosed separately in a plastic bag and then detached from the stem of the plant with a scissors. The bags were brought to the laboratory and aphids were identified to species. Mummies were placed separately in small plastic vials, covered with muslin cloth, tied with rubber band, and labelled with details of collection date and the serial number of the terminal 10cm shoot and leaves. Next, the plastic vials were kept under laboratory conditions of 26±5°C, 65±5% RH and 16L: 8D until emergence of the adult parasitoid/hyperparasitoid.

3.2.4 Data analysis

The mean total number of aphids, percentage of mummified aphids to the total number of aphids (Tomanovic *et al*., 1996; Kavallieratos *et al*., 2002a, b, 2004), the
percentage of hyperparasitized mummies to the total number of mummies and percentage of *D. rapae, A. colemani* and *P. aphidis* to the total number of mummies per terminal 10cm shoot and middle and lower leaf were calculated per sampling date. Analysis of variance was done after transformation of the data $y_{trans} = \sqrt{(y + 0.5)}$ to normalise variances and standardise means using the statistical package SPSS 16.0.

One way ANOVA was used to test the significance of differences in the total number of: (a) *L. erysimi* individuals present among the three sampling subunits, viz., terminal 10cm shoot, middle and lower leaf of the plants; (b) species of aphidiine parasitoids (*D. rapae* and *A. colemani*) which emerged from each sampling subunit; (c) *D. rapae* individuals which emerged from each sampling subunit; (d) *A. colemani* individuals which emerged from each sampling subunit; (e) hyperparasitoids which emerged from each sampling sub unit during the entire period of the study for each year. Means were finally compared with the Tukey-Kramer (HSD) test (at $P = 0.05$)

### 3.2.5 Augmentation of specimens

All the specimens of *D. rapae, A. colemani* and *P. aphidis* will be augmented to National Pusa Collection (NPC), Division of Entomology, IARI, New Delhi.
Redescription of Diaeretiella rapae (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) with emphasis on the variability of morphometric characters

ABSTRACT

Characters traditionally used for identification of Diaeretiella rapae (M’Intosh), a common polyphagous parasitoid of several aphids were seen to be more variable than previously supposed. Around 300 specimens were collected from nine different locations in India and examined. Variability in the following morphological characters were recorded viz., number of antennal segments in males and females; number of lateral carina on anterolateral area of petiole; central propodeal areola; tentorial index; gena width; dimensions of F1 and F2; ratio of pterostigma width to pterostigma length; tergite I length. The redescription was further strengthened with the addition of several new characters viz., propodeal characters namely, median longitudinal carina length and central areola width along with several new wing parameters like, metacarp (R1) length, radial length, M basal length, distance between tip of r and apex of wing, distance between tip of r and base or R1, distance between base of stigma and r, M+Cu length and maximum width of the first sub discal cell etc. Statistical analysis was used to further quantify the range of variation observed.

Keywords: Aphidiinae, Diaeretiella rapae, morphological variability, redescription, systematics.

4.1 Introduction

Crop protection against insect pests depends largely on application of chemical insecticides. These continue to provide reliable and cost effective control but cause concern because they can harm non-target organisms, such as parasitoids and pollinators. Crop management systems for the future, however, must contain sustainability and
environmental acceptability to safety of both social and economic demands, they should be high-yielding yet energy efficient, providing a good economic net return. Consequently, there is now considerable emphasis on minimizing pesticides applications within IPM systems and enhancing the use of natural biocontrol agents. Consequently interest in the control potential of hymenopterous parasitoids, particularly in the most cosmopolitan species *Diaeretiella rapae* has grown significantly.

*D. rapae* was originally described by McIntosh in 1855 as *Aphidius rapae*. Genus *Diaeretiella* was erected by Stary (1960) with the following characters: head transverse, as wide as or wider than thorax at tegulae; antennae filiform, with variable number of segments (12 to 18); eye of medium size; mandibles bidentate. Notaulices developed on the fore part of mesoscutum. Propodeum distinctly areolated. Fore wing with pterostigma triangular; metacarp longer than width of pterostigma; radial vein developed, not longer than ⅔ of its possible length; otherwise venation effaced beyond basal cell towards the apex except cubital cell 2 and indicated part of cubital vein. Hind wing with complete basal cell. Abdomen of female lanceolate; ovipositor sheaths and ovipositor straight or slightly curved upwards, sparsely haired and the species *rapae* was transferred to this genus. However, these characters were found to be much more variable than has been previously supposed. This paper shows the extent of variation occurring in the various characters and evaluates their reliability for making taxonomic distinctions.

### 4.2 Material and Methods

#### 4.2.1 Sources of biological materials

200 females and 50 males were collected from nine different places of India (Table 4.1). Collections were made within a weeks time period in order to maintain homogeneity of samples. Due care was taken to label these samples with data like host name, date of collection, locality and details of collector before suitably storing.

#### 4.2.2 Preparation and Preservation

Adult parasitoids were preserved in 70% ethyl alcohol and later identified to the species using essential diagnostic characters under Leica MZ12 stereo zoom microscope with suitable illumination. Specimens in intact conditions suitable for morphometric analysis were selected and dry mounted on rectangular paper tags using a fine brush and
needle. Each specimen was labelled with details of host, date of collection, locality and collector’s name and placed in insect boxes for further studies.

4.2.3 Selection of samples

These dry mounted specimens were individually examined under Leica WILD HEERBRUGG compound stereo zoom microscope at magnification of 40x to 100x to confirm uniformity and suitability for morphometrics. The physical condition like cleanliness, completeness and amenability for measurement were the criteria utilized here for selection of specimens. Such good female specimens of nearly uniform and stable conditions with least variability were randomly selected and individually numbered before undertaking morphometric analyses.

4.2.4 Selection of characters

The available taxonomic literature on the descriptions or redescriptions of *D. rapae* and its diagnostics at different hierarchical levels were consulted for selection of characters. Studies by M’Intosh (1855), Mukerji and Chatterjee (1950), Stary (1961) and Akthar et al. (2011) on the taxonomy and morphometrics of *D. rapae* were taken into consideration. Literature indicates that propodeal and fore wing characters are of extreme importance in identification of *D. rapae*, and hence all characters available on propodeum and fore wing were taken into consideration along with addition of some new characters (Table 4.2). Forty quantitative characters were measured in 167 specimens from eleven populations of *D. rapae* which were crucial in defining *D. rapae* at all its taxonomic levels. The quantitative characters were of two kinds: meristic and ratios (Table 4.2 and Fig. 4.1-4.8). The use of ratios in studies of numerical taxonomy as measures of shape was recommended by Hills (1978), Dodson (1978) and Tomnic et al. (2005). We used logarithmic transformations of our data performed on the log(x+1) of both meristic and ratio characters (Hills, 1978).

4.2.5 Study and measurement of characters

While studying the specimens under microscope, care was taken to ensure that each specimen are always in a level plane or set uniformly every time at the same angle. While doing so, the precautions to be taken at the time of microscopic examinations of taxonomic characters by different workers were kept in mind. All the measurements were taken under Leica WILD HEERBRUGG compound stereo zoom microscope at
magnification of 130x except, number of lateral carinae on tergite I, which was counted
at magnification of 320x fitted with ocular micrometer. The measurements in terms of
ocular values thus converted into mm values using a stage micrometer. Such values were
tabulated and then subjected to further analyses.

4.2.6 Data analysis

The measurement of taxonomic characters of *D. rapae* was tabulated separately
for different populations. Morphometric analyses were performed using MS Windows
(version 2007) operated MS Excel (version 2007).

4.3 Results

Redescription of genus *Diaeretiella* Stary


**Type Species**: *Aphidius rapae* M’Intosh, 1855 by original designation.


**Diagnosis**: Head transverse, as wide as or wider than mesosoma at tegulae; antennae
filiform, with variable number of segments (13 to 15 segments in female and 16 to 17
segments in male); eyes medium sized; mandibles bidentate. Notauli developed on the
ascending fore part of mesoscutum; fore wing: stigma triangular; metacarp (*R<sub>1</sub>*) 2.7x
longer than maximum width of stigma; radial sector developed, about 0.25x its possible
length, never reaches margin; marginal cell incomplete; otherwise venation effaced
beyond basal cell towards apex except cubital cell 2 and indicated part of cubital vein.
Propodeum distinctly areolated with a narrow central areola; Metasoma of female
lanceolate, rounded at apex in male; ovipositor sheaths and ovipositor slightly curved
upwards, sparsely hairy at apex.

**General Distribution**: Cosmopolitan.

**Bionomics**: Exclusively parasites of aphids with pupation occurring inside parasitized
aphid.
Note: This genus is related to *Aphidius* Nees, but differs from it due to further reduction in wing venation.

**Redescription of *Diaeretiella rapae* (M’Intosh, 1855)**

(Fig. 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8)

?*Aphidius vulgaris* Bouche, 1834: 161-162.

*Aphidius rapae* M’Intosh, 1855: 194.

*Aphidius (Trionyx) rapae* Curtis, 1860: 73-74.

*Diaeretus chenopodii* Foerster, 1867: 125.

*Trioxys piceus* Cresson, 1880: 260.

*Lipolexis chenopodiaphidis* Ashmead, 1889: 671.


*Diaeretus nipponensis* Viereck, 1911: 182.


*Diaeretus napus* Quilis, 1931: 71-72.

*Diaeretus croaticus* Quilis, 1934: 8-9.

*Diaeretus plesiorapae* Blanchard, 1940: 45-48.

*Diaeretus aphidum* Mukherji and Chatterjee, 1950: 4-6.

**Female:**

**Colour:** Rather variable. Head black, face sometimes yellow, clypeus and mouthparts yellow to brownish-yellow; antennae brownish-black, with a lighter ring between pedicel and flagellar segment I, often scape, pedicel and base of flagellar segment I yellow. Thorax black; prothorax sometimes yellow to brownish-yellow; wing venation brown; tegulae brown; legs brown to dark brown, lower part of coxae, trochanters, base of tibiae and tarsi light brown to yellow. Abdomen brown to brownish-black, tergite I yellow to dark brown, base of tergite II and suture between tergites II and III similar colored.
Head transverse to subquadrate, 2.02X wider than long, smooth, shiny, sparsely hairy, 1.19x wider than mesosoma at tegulae; occiput margined. Temple width 0.84x transverse eye diameter; gena width 0.45x longitudinal eye diameter; intertentorial line 0.23x head width, tentorial index 0.36; clypeus transverse, oval, convex, margined frontally, smooth shiny with six to eight long hairs, separated from face by shallow grooves; mandibles broad basally, 0.57x gena at base, bifurcate apically, uniformly narrowed towards apex. Eyes prolongately oval of medium size, strongly convergent towards clypeus. Ocelli medium sized, forming an equilateral triangle; interorbital distance 2.99x interocellar distance; oculocellar distance 1.25x interocellar distance. Antenna mostly 14-segmented (rarely 13 or 15), filiform, about as long as head, thorax and tergite I combined; antennal socket ocular line 0.61x inter antennal distance; scape and pedicel almost sub-equal to their maximum length and width; length of flagellar segment I and II subequal; length of flagellar segment I 2.74x its maximum width; length of flagellar segment II 2.77x its maximum width; flagellar segment II 0.77x length of last flagellar segment; apical segment 3.58x as long as its maximum width.

Mesosoma smooth, shiny, sparsely hairy; mesoscutum falling almost vertically to pronotum, without covering it when viewed from side; mesonotum length 1.30x width at tegulae; mesopleuron width 0.31x mesosoma length; notauli distinct anteriorly, narrow, crenulate but effaced on disc, fore margin slightly prominent. Fore wing hyaline, length 2.52x its maximum width; stigma triangular, 0.26x wider than long; metacarp (R₁) 1.23x longer than radial (r) vein; ‘r’ arising from about basal 0.45 of stigma, about 0.65 as long as stigma; marginal cell incomplete; r-m and m-cu absent; basal part of M 0.41xas long as maximum width of fore wing. Hind wing with complete basal cell, SC+R prominent. Legs with hind coxa 0.5x as long as hind femur; length of hind basitarsus 0.42x length of hind tibia; hind coxa 3x maximum width of mesopleuron; length of hind tarsal segment II to V gradually decreasing in length.

Propodeum with small narrow central pentagonal areola of variable shape, sometimes subdivided basally; propodeum 0.54x as wide as length of mesonotum ; maximum width of central areola 0.12x of maximum mesonotal width at tegulae; disc of areola smooth, shiny, upper lateral areola with 3 to 6, lower with 2 to 3 hairs; transverse carinae arising
from either side of mid lateral part of areola reaching spiracles; median carina originating on top of areola running up to postscutellum, median carina 2.32x longer than dorsal carina on first tergum; few small carinae present around basal region of areola; propodeal spiracle located on lateral propodeal margin.

**Metasoma** lanceolate, longer than head and mesosoma combined; tergum I slender, costulate, slightly granulate, dilated towards apex, about 2.40x longer than wide at spiracles, 0.25x as long as metasoma; dorsal longitudinal carina 0.16x tergum I length; lateral longitudinal carina prominent, variable in number (9 to 15), more or less coarsely rugose anteriorly, posterior 0.25 smooth; spiracular tubercles hardly visible, situated somewhat before mid-line of tergite I; tergum II smooth, shiny, sparsely hairy.

**Genitalia:** Ovipositor sheath 0.36x as long as tergum I, slightly curved upwards, about 1.48x longer than its maximum basal width, sparsely hairy, bearing 4 to 6 setae; ovipositor with two pair of valves, curved upwards, ending in a pointed tip.

**Body length:** About 2.05±0.16 mm.

**Measurements (mm±SD):** **Head:** tenteriocular line 0.03±0.01, intertentorial line 0.10±0.0, inter-ocellar line 0.12±0.01, antennal socket ocular line 0.03±0.003, temple width 0.12±0.01, gena width 0.9±0.01, head length 0.21±0.02, head width 0.42±0.02, interorbital distance 0.26±0.03, interocellar distance 0.09±0.01, oculocellar distance 0.11±0.01, mandible width at base 0.05±0.01, longitudinal eye diameter 0.22±0.01, transverse eye diameter 0.15±0.01, inter antennal distance 0.04±0.01, scape length 0.06±0.01, pedicel length 0.06±0.01, flagellomere I length 0.10±0.01, flagellomere II length 0.10±0.01, last flagellomere length 0.13±0.01, scape width 0.05±0.01, pedicel width 0.05±0.01, flagellomere I width 0.04±0.01, flagellomere II width 0.04±0.01, last flagellomere width 0.04±0.01.

**Mesosoma:** pronotum length 0.04±0.01, mesonotum length 0.47±0.04, mesonotum width at tegulae 0.37±0.03, mesosomalength 0.66±0.07, mesopleuron width 0.20±0.01, propodeum width 0.26±0.04, median longitudinal carina length 0.09±0.01, central areola width 0.04±0.01.
Wings: fore wing length 1.76±0.17, maximum width of fore wing 0.67±0.10, length of costa + subcosta 0.65±0.05, stigma length 0.33±0.04, stigma width 0.10±0.01, metacarp (R1) length 0.26±0.03, length of radial (r) 0.22±0.03, length of M basal 0.28±0.03, distance between tip of rand apex of wing 0.59±0.04, distance between tip of r and base or R1 0.18±0.03, distance between base of stigma and r 0.15±0.01, M+Cu length 0.44±0.04, maximum width of the first sub discal cell 0.03±0.01, 1Cualength0.19±0.03, length of fore wing fold 0.26±0.03, width of fore wing fold 0.02±0.01, distance between 1Cub and fore wing fold 0.09±0.03.

Legs: hind coxa length 0.19±0.01, hind femur length 0.40±0.04, hind tibia length 0.59±0.05, hind basitarsus length 0.25±0.03.

Metasoma: tergum I length 0.26±0.02, tergum I width 0.10±0.01, length of dorsal carina on tergum I 0.04±0.01, gaster length 1.07±0.07, ovipositor sheath length 0.10±0.01, ovipositor sheath width at base 0.06±0.01.

Male: Similar to female except the following: antenna 16 to 17-segmented; black, mouthparts and tergite I brownish-yellow; legs brownish-black; otherwise, coloration as in female; tergite I nearly parallel-sided.

Mummy: Dark shining brown; adult emerges from posterior end by cutting a smooth round hole.

Distributions: Cosmopolitan (Stary, 1961); Assam (Rao et al., 1969); Himachal Pradesh (Saha et al., 1982; Batra and Wahdi, 1962); Jammu and Kashmir (Bhagat and Ahmad, 1991; Rao et al., 1970; Takada and Rishi, 1980); Karnataka (Sethumadhavan and Dharmadhikari, 1969; Rao et al., 1969, 1970); Meghalaya (Stary and Ghosh, 1975, 1978); Punjab (Atwal et al., 1969, 1971); Sikkim (Agarwala et al., 1980); Uttar Pradesh (Anonymous, 1968; Rao et al., 1970; Singh et al., 1999; Das and Chakrabarti, 1990; Akhtar et al., 2006).

Material examined: 200♀♀ and 50 ♂♂: INDIA: 10 ♀♀ and 7 ♂♂: Aligarh, Uttar Pradesh, 06. III. 2010, parasitic on L. erysimi on cabbage, Coll. Mir Samim Akhtar; 15 ♀♀ and 5 ♂♂: Almora, Uttarakhand, 07. III. 2010, parasitic on L. erysimi on cabbage,

**Host:** *Aphis craccivora* (Atwal et al., 1969) on *Solanum tuberosum* (Bhagat and Ahmad, 1991); *A. gossypii* (Sethumadhavan and Dharmadhikari, 1969); *A. ruborum longisetosus* on *Punica granatum* (Saha et al., 1982); *Brachycaudus helichrysi* on *Prunus amygdalus* (Das and Chakrabarti, 1990); *Brevicoryne brassicae* (Rao, et al., 1970; Sethumadhavan and Dharmadhikari, 1969) on Brassicaceae plant (Bhagat and Ahmad, 1991), Cabbage (Batra and Wahdi, 1962), *Brassica nigra*, *B. oleracea*, *Raphanus sativus* (Saha et al., 1982), *Brassica* spp. (Stary and Ghosh, 1975), *Descorina sofia* (Takada and Rishi, 1980), *Raphanus sativus* (Singh et al., 1999); *Hayhursta atrilicus* (Sethumadhavan and Dharmadhikari, 1969) on *Chenopodium album* (Das and Chakrabarti, 1990); *Lipaphis erysimi* (Atwal et al., 1971; Kundu et al., 1966; Rao et al., 1969; Subhrani et al., 2006) on *Brassica campestris* (Bhagat and Ahmad, 1991) var. *cauliflower* (Singh et al., 1999), *Brassica junce* var. M27, rugose, capitata (Subhrani et al., 2006), *B. napus* (Stary and Ghosh, 1978), *B. oleracea* (Agarwala et al., 1980), Mustard (Akhtar et al., 2006), *Brassica* spp. (Saha et al., 1982); *Macrosiphoniella pseudoartemisae* on *Brassica* spp. (Saha et al., 1982); *Metopolophium* (Metopolophium) *drihodun* (Anonymous, 1968) on Grass (Saha et al., 1982); *Myzus persicae* (Atwal et al., 1969; Rao et al., 1970; Sethumadhavan and Dharmadhikari, 1969) on *Mirabilis jalapa*, *Raphanus sativa* (Saha et al., 1982), *Descorina sofia* (Takada and Rishi, 1980); *Sitobion avenae* eleusinae
(Atwal et al., 1969), *S. rosaeformis* on *Rosa* sp. (Saha et al., 1982); Unidentified Aphids (Bhagat and Ahmad, 1991)

**Host-specificity:** *D. rapae* is polyphagous, but it prefers aphids of subfamily Myzinae, and aphids on cruciferaceous and chenopodiaceous plants.

**Remarks:** Antenna is peculiar with complete and incomplete segmentation of last flagellomere. Antenna is 13 or 14 segmented in females depending upon the level of subdivision of the last antennal segment. Central propodeal areola shows considerable variability in width and shape, sometimes even subdivided into 2 to 3 subareola, hence, propodeal areola character should be used carefully in a diagnostic key.

**4.4 Discussion**

Recognition of species is of utmost importance for IPM and biological control programmes as species can have different host ranges and host preferences. The data of the present studies suggests that the morphological basis upon which the nominal species has been distinguished is unsatisfactory.

Although while redescribing *D. rapae*, Stary (1961) gave morphometric details of a few taxonomic characters like “… temple as wide as transverse eye-diameter; gena as wide as 1/3 to 1/7 of longitudinal eye-diameter; tentorio-ocular line 1/4 to 1/6 as long as intertentorial line; F1 and F2 of equal length, 2.5 times as long as wide; tergite I about 3 to 3.5 times longer than wide at spiracles…” but he did not quantify many important characters like: length of last flagellomere/length of flagellomere I; length of last flagellomere/width of last flagellomere; tentorio-ocular line/inter tentorial line; length of median longitudinal carina on propodeum/length of dorsal carina on first tergum; width of central areola/width of mesopleuron; length of fore wing/maximum width or fore wing; width of stigma/length of stigma; length of ovipositor sheath/length of tergite I; length of ovipositor sheath/width of ovipositor sheath at base and number of lateral carinae on tergite I.

Present studies show that the number of antennal segments varied by two in males and three in females. The number of lateral carina on anterolateral area of petiole varied
widely between nine to fifteen. Central propodeal areola shows considerable variability in width and shape, sometimes is even subdivided into 2 to 3 sub areolas. The tentorial index ranges from 0.27-0.45 compared to previously published range of 0.17-0.25. Gena was observed to be as wide as 0.42-0.46 of longitudinal eye-diameter as compared to the previously published ranges of 0.14-0.20. Previously published description recorded F1 and F2 of equal length and 2.5 times as long as wide but the present studies quantify F1 and F2 as 2.77±0.18 mm and 2.76±0.17 mm. Ratio of width of pterostigma to length of pterostigma ranges from 0.26-0.34, as compared to previously published range of 0.20-0.25. Tergite I was previously described as about 3-3.5 times longer than width at spiracles, but presently it was recorded to vary in the range of 2.36-2.73.

The description of *D. rapae* was further strengthened with the following additional characters, *viz.*, two new propodeal characters namely, median longitudinal carina length and central areola width; several new wing parameters like, metacarp (*R*1) length, radial length, M basal length, distance between tip of r and apex of wing, distance between tip of r and base or *R*1, distance between base of stigma and r, M+Cu length and maximum width of the first sub discal cell etc to quantify the variability in the propodeal region and the wing.
Fig. 4.1: General morphology of ♀ D. rapae
Fig. 4.2: General morphology of $\delta$ D. rapae
Fig. 4.3: A, B. Antenna; C. Mesosoma
Fig. 4.4: Fore and hind wing of *D. rapae*
Fig. 4.5: A. Fore leg; B. Middle leg; C. Hind leg; D. Last tarsal segment
Fig. 4.6: A. Propodeum; B. Tergite I
Fig. 4.7: A. ♀ Genitalia; B. ♂ Genitalia
Fig. 4.8: Character code for fore wing measurements of *D. rapae*
Fig. 5.1 Hierarchical cluster between the eleven *D. rapae* populations used in the study (using AVERAGE clustering method).
Table 4.1 List of population codes and localities of the material examined

<table>
<thead>
<tr>
<th>Serial Number</th>
<th>Population code</th>
<th>Number of specimens (females)</th>
<th>Aphid-plant associations</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ALI CAB</td>
<td>10</td>
<td><em>Lipaphis erysimi</em> on Cabbage</td>
<td>Aligarh</td>
</tr>
<tr>
<td>2</td>
<td>ALM CAB</td>
<td>15</td>
<td><em>L. erysimi</em> on Cabbage</td>
<td>Almora</td>
</tr>
<tr>
<td>3</td>
<td>ALM MUS</td>
<td>15</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Almora</td>
</tr>
<tr>
<td>4</td>
<td>BAR CAB</td>
<td>9</td>
<td><em>L. erysimi</em> on Cabbage</td>
<td>Barapani</td>
</tr>
<tr>
<td>5</td>
<td>DEL CAB</td>
<td>20</td>
<td><em>L. erysimi</em> on Cabbage</td>
<td>Delhi</td>
</tr>
<tr>
<td>6</td>
<td>DEL MUS</td>
<td>24</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Delhi</td>
</tr>
<tr>
<td>7</td>
<td>DER MUS</td>
<td>10</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Deradhun</td>
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<tr>
<td>8</td>
<td>IND MUS</td>
<td>18</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Indore</td>
</tr>
<tr>
<td>9</td>
<td>NAI MUS</td>
<td>10</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Nainital</td>
</tr>
<tr>
<td>10</td>
<td>PANT CAB</td>
<td>20</td>
<td><em>L. erysimi</em> on Cabbage</td>
<td>Pantnagar</td>
</tr>
<tr>
<td>11</td>
<td>PANT MUS</td>
<td>16</td>
<td><em>L. erysimi</em> on Mustard</td>
<td>Pantnagar</td>
</tr>
<tr>
<td>Character code</td>
<td>Type</td>
<td>Description</td>
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<td></td>
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<tr>
<td>----------------</td>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. NAS</td>
<td>Meristic</td>
<td>Number of antennal segments</td>
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<td></td>
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<tr>
<td>2. LS/LP</td>
<td>Ratio</td>
<td>Length of scape/length of pedicle</td>
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<tr>
<td>3. LF1/LF2</td>
<td>Ratio</td>
<td>Length of flagellomere I/length of flagellomere II</td>
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<td></td>
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<tr>
<td>4. LFL/LF1</td>
<td>Ratio</td>
<td>Length of last flagellomere/length of flagellomere I</td>
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<td>5. WS/ WP</td>
<td>Ratio</td>
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<td>6. LF1/WF1</td>
<td>Ratio</td>
<td>Length of flagellomere I/width of flagellomere I</td>
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<tr>
<td>7. LF2/WF2</td>
<td>Ratio</td>
<td>Length of flagellomere II/width of flagellomere II</td>
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<td></td>
</tr>
<tr>
<td>8. LFL/WFL</td>
<td>Ratio</td>
<td>Length of last flagellomere/width of last flagellomere</td>
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</tr>
<tr>
<td>9. OT/IT</td>
<td>Ratio</td>
<td>Tenterio-ocular line/inter tentorial line</td>
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<tr>
<td>10. AO/DAT</td>
<td>Ratio</td>
<td>Antennal socket ocular line/distance between antennae</td>
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<td></td>
</tr>
<tr>
<td>11. TW/GW</td>
<td>Ratio</td>
<td>Width of temple/width of gena</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. LH/WH</td>
<td>Ratio</td>
<td>Length of head/width of head</td>
<td></td>
<td></td>
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<tr>
<td>13. IOR/IOC</td>
<td>Ratio</td>
<td>Intero orbital distance/intero ocellar distance</td>
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<tr>
<td>14. OOC/IOC</td>
<td>Ratio</td>
<td>Oculo ocellar distance/intero ocellar distance</td>
<td></td>
<td></td>
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<tr>
<td>15. WM/GW</td>
<td>Ratio</td>
<td>Mandible width at base/width of gena</td>
<td></td>
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</tr>
<tr>
<td>16. LED/TED</td>
<td>Ratio</td>
<td>Longitudinal eye diameter/transverse eye diameter</td>
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<td>17. LPN/LMN</td>
<td>Ratio</td>
<td>Length of pronotum/length of mesonotum</td>
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<td>18. LMN/WMT</td>
<td>Ratio</td>
<td>Length of mesonotum/width of mesonotum at tegulae</td>
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<td>19. WMP/LMES</td>
<td>Ratio</td>
<td>Width of mesopleuron/length of mesosoma</td>
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<td></td>
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<tr>
<td>20. WPR/LMN</td>
<td>Ratio</td>
<td>Width of propodeum/length of mesonotum</td>
<td></td>
<td></td>
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<tr>
<td>21. LMC/LCT1</td>
<td>Ratio</td>
<td>Length of median longitudinal carina on propodeum/length of dorsal carina on</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>first tergum</td>
<td></td>
<td></td>
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<tr>
<td>22. WCA/WMP</td>
<td>Ratio</td>
<td>Width of central areola/width of mesopleuron</td>
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<tr>
<td>23. LHC/LHF</td>
<td>Ratio</td>
<td>Length of hind coxa/length of hind femur</td>
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<td>24. WMP/LHT</td>
<td>Ratio</td>
<td>Width of mesopleuron/length of hind tibia</td>
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<tr>
<td>25. LHB/LHT</td>
<td>Ratio</td>
<td>Length of hind basitarsus/length of hind tibia</td>
<td></td>
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<tr>
<td>26. LFW/WFW</td>
<td>Ratio</td>
<td>Length of fore wing/maximum width or fore wing</td>
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<td></td>
</tr>
<tr>
<td>27. LCSC/LFW</td>
<td>Ratio</td>
<td>Length of costa + subcosta/length of fore wing</td>
<td></td>
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<tr>
<td>28. WSt/LSt</td>
<td>Ratio</td>
<td>Width of stigma/length of stigma</td>
<td></td>
<td></td>
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<tr>
<td>29. LR1/LrRs</td>
<td>Ratio</td>
<td>Length of metacarp (R1)/length of r and Rs</td>
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<td></td>
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<td>30. LMB/WFW</td>
<td>Ratio</td>
<td>Length of basal part of M/maximum width or fore wing</td>
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<tr>
<td>31. DTA/LFW</td>
<td>Ratio</td>
<td>Distance between tip of r and Rs and apex of wing/length of fore wing</td>
<td></td>
<td></td>
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<tr>
<td>32. DTR1/WFW</td>
<td>Ratio</td>
<td>Distance between tip of r and Rs and base or R1/maximum width or fore wing</td>
<td></td>
<td></td>
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<tr>
<td>33. DTR1/DTA</td>
<td>Ratio</td>
<td>Distance between tip of r and Rs and base of R1/distance between tip of r</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>and Rs and apex of wing</td>
<td></td>
<td></td>
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<tr>
<td>34. DSrRS/LSt</td>
<td>Ratio</td>
<td>Distance between base of stigma and r and Rs/length of stigma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35. LMCu/L1CuA</td>
<td>Ratio</td>
<td>Length of M+Cu/length of 1Cu</td>
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<td></td>
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<tr>
<td>36. LT1/WT1</td>
<td>Ratio</td>
<td>Length of tergite I/width of tergite I</td>
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<tr>
<td>37. LT1/LA</td>
<td>Ratio</td>
<td>Length of tergite I / length of abdomen</td>
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<td></td>
</tr>
<tr>
<td>38. LOS/LT1</td>
<td>Ratio</td>
<td>Length of ovipositor sheath/ length of tergite I</td>
<td></td>
<td></td>
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<tr>
<td>39. LOS/WOS</td>
<td>Ratio</td>
<td>Length of ovipositor sheath/width of ovipositor sheath at base</td>
<td></td>
<td></td>
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<tr>
<td>40. NLCT1</td>
<td>Meristic</td>
<td>Number of lateral carinae on tergite I</td>
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</table>
Comparison of eleven populations of *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) from India

**Abstract**

Eleven populations of *Diaeretiella rapae* reared from parasitized *Lipaphis erysimi* were collected from nine different places of India among which six populations were collected on mustard from Almora, Delhi, Deradhun, Indore, Nainital and Pantnagar and five populations parasitizing *L. erysimi* were collected on cabbage from Aligarh, Almora, Barapani, Delhi and Pantnagar. Analyses of measurements showed that, three ratio characters viz. LF1/WF1 (Length of flagellomere I/width of flagellomere I), WMP/LMES (Width of mesopleuron/length of mesosoma) and LMCu/L1CuA (Length of M+Cu/length of 1CuA) had heavier principal component weights in the first principal component and served as the main contributing variables in the diagnostic differentiation of the populations. Following cluster analysis two groups were formed: Group 1 dominated by population from plains and Group 2 dominated by population from higher altitudes. *D. rapae* reared from *L. erysimi* infesting cabbage in Aligarh and Delhi were found as the most closely related populations in the first group, with population from Pantnagar mustard population joining as a sister group to the two. In the second group population from Nainital mustard and Pantnagar cabbage were placed as the most closely related populations, with Almora mustard joining as a sister group to them. From our study it was hypothesized that being widely distributed in different parts of India, with wide host range, formation of host races as well as geographical races in *D. rapae* appears to be a remote possibility although some variability may be observed between the populations of plain and hill region.

**Keywords:** Morphometry, *Diaeretiella rapae*, Aphidiinae, systematic, host race, morphological variability
5.1 Introduction

Aphids as pests are known worldwide. Their biological peculiarities along with extensive monoculture and indiscriminate use of agricultural chemicals etc. have complicated matters further which have in turn stimulated research on their biological control. Oilseed *Brassicas* are among the major oilseed crops cultivated in India and around the world. India produces about 11.3% of the world’s rapeseed-mustard (Damodaram and Hegde, 2002). Among the oilseeds mustard is one of the major sources of edible oil for human consumption. *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae) is the main aphid pest infesting mustard in several areas in India and its infestation by *L. erysimi* reduces directly both yield and quality of the product and causes upto 83% losses in mustard (Mandal et al., 1994).

Aphid parasitoids belonging to the braconid subfamily Aphidiinae (Hymenoptera) are solitary endoparasitoids of aphids (Stary 1970, 1988). Many species are important natural enemies of pest aphids and are often used in biological control programs worldwide (Carver 1989; Hughes 1989; Hagvar and Hofsvang 1991).

*Diaeretiella rapae* was originally described by McIntosh in 1855 as *Aphidius rapae* which was brought under the genus *Diaeretiella* by Stary (1961). *D. rapae* has been recorded from several aphid taxa viz., *Aphis ruborum longisetosus, Aphis craccivora, Aphis gossypii, Brachycaudus helichrysi, Brevicoryne brassicae, Hayhurstia atriplicis, Lipaphis erysimi, Macrosiphiella pseudoartemisae, Macrosiphum (Metopolophium) driodun, Macrosiphum (Sitobion) rosaeformis, Myzus persicae, Sitobion avenae eleusinae* are recorded from India (Dey and Akhtar, 2007). Being a polyphagous and cosmopolitan parasitoid of aphids it is considered as an important control agent for aphid pests in a variety of agricultural and horticultural ecosystems (Hagvar and Hofsvang, 1991; Pike et al., 1999; Dogra et al., 2003; Dhiman, 2007; Akhtar et al., 2010). It was exported from China to USA for biological control of *Diuraphis noxia* (Mordwilko), a pest of small grains (Bernal and Gonzalez, 1997).

While redescribing *D. rapae*, Stary (1961) gave morphometric details of few taxonomic characters like “… temple as wide as transverse eye-diameter; gena as wide as 1/3 to1/7 of longitudinal eye-diameter; tentorio-ocular line 1/4 to 1/6 as long
as intertentorial line; F1 and F2 of equal length, 2.5 times as long as wide; tergite I about 3 to 3.5 times longer than wide at spiracles…"etc. but failed to quantify variability of several other important characters.

While working on morphological variability of several biotypes of *Ephedrus plagiator* (Nees, 1811), Tomic et al. (2005), recomended the use of ratios in studies of numerical taxonomy as measures of shape following the principal laid by Hills (1978) and Dodson (1978). Although considerable work has been done on the morphometrics of Braconidae (Tomic et al., 2005; Billah et al., 2008), morphological variability in intraspecific population of *D. rapae* has got little attention. Akhtar et al., (2011) redescribed *D. rapae* and presented a brief account of morphological variability among four populations from India on the basis of ten character ratios, using numerical methods. His investigation indicated that the specimens reared from different localities showed clear distinction from other populations.

The knowledge of the taxonomy and diversity of *D. rapae* has great importance for successful biological control programs. The aim of the present paper is a taxonomic study using numerical methods of several populations of *D. rapae* from different parts of India. In our study we have used taxonomic characters with a high content of diagnostic information.

5.2 MATERIAL AND METHODS
5.2.1 Sources of biological materials

Eleven samples of *Diaeretiella rapae* populations were collected from nine different places of India (Table. 4.1). Six populations parasitizing *Lipaphis erysimi* on mustard were collected from Almora (23°36'06"N, 79°39'16"E; elevation 1456 m), Delhi (28°04'01"N, 77°09'06"E; elevation 223 m), Dehradun (30°19'17"N, 78°01'55"E; elevation 652 m), Indore (22°43'31"N, 75°51'56"E; elevation 511 m), Nainital (29°23'52"N, 79°26'08"E; elevation 2031 m) and Pantnagar (29°01'20"N, 79°29'15"E; elevation 234 m). Five populations parasitizing *L. erysimi* on cabbage were also collected from Aligarh (27°54'01"N, 78°04'58"E; elevation 191 m), Almora (23°36'06"N, 79°39'16"E; elevation 1456 m), Barapani (25°43'11"N, 91°58'43"E; elevation 906 m), Delhi (28°04'01"N, 77°09'06"E; elevation 223 m) and Pantnagar (29°01'20"N, 79°29'15"E; elevation 234 m). Collections were made within a weeks time period in order to maintain homogeneity of samples. Due care was taken to label
these samples with data like host name, date of collection, locality and details of collector before suitably storing.

5.2.2 Preparation and Preservation

Adult parasitoids were preserved in 70% ethyl alcohol and later identified to the species using essential diagnostic characters under Leica MZ12 stereo zoom microscope with suitable illumination. Specimens in intact conditions suitable for morphometric analysis were selected and dry mounted on rectangular paper tags using a fine brush and needle. Each specimen was labelled with details of host, date of collection, locality and collector’s name and placed in insect boxes for further studies.

5.2.3 Selection of samples

These dry mounted specimens were individually examined under Leica WILD HEERBRUGG compound stereo zoom microscope at magnification of 40x to 100x to confirm uniformity and suitability for morphometrics. The physical condition like cleanliness, completeness and amenability for measurement were the criteria utilized here for selection of specimens. Such good female specimens of nearly uniform and stable conditions with least variability were randomly selected and individually numbered before undertaking morphometric analyses.

5.2.4 Selection of characters

The available taxonomic literature on the descriptions or redescriptions of *D. rapae* and its diagnostics at different hierarchical levels were consulted for selection of characters. Studies by M’Intosh (1855), Mukerji and Chatterjee (1950), Stary (1961) and Akthar *et al.*, (2011) on the taxonomy and morphometrics of *D. rapae* were taken into consideration. Literature indicates that propodeal and fore wing characters are of extreme importance in identification of *D. rapae*, and hence all characters available on propodeum and fore wing were taken into consideration along with addition of some new characters (Table 4.2). Forty quantitative characters were measured in 167 specimens from eleven populations of *D. rapae* which were crucial in defining *D. rapae* at all its taxonomic levels (Table 4.2 and Fig. 4.1-4.8). The quantitative characters were of two kinds: meristic and ratios (Table 4.2). The use of ratios in studies of numerical taxonomy as measures of shape was recommended by Hills (1978), Dodson (1978) and Tomic *et al.* (2005). We used logarithmic
transformations of our data performed on the log(x+1) of both meristic and ratio characters (Hills 1978). All the 40 quantitative characters with descriptive statistics parameters and summary statistics of logarithmic transformed data have been listed in Table 5.1.

5.2.5 Study and measurement of characters

While studying the specimens under microscope, care was taken to ensure that each specimen are always in a level plane or set uniformly every time at the same angle. While doing so, the precautions to be taken at the time of microscopic examinations of taxonomic characters by different workers were kept in mind. All the measurements were taken under Leica WILD HEERBRUGG compound stereo zoom microscope at magnification of 130x except, number of lateral carinae on tergite I, which was counted at magnification of 320x fitted with ocular micrometer. The measurements in terms of ocular values thus converted into mm values using a stage micrometer. Such values were tabulated and then subjected to further analyses.

5.2.6 Data analysis

The measurement of taxonomic characters of *D. rapae* was tabulated separately for different populations. Morphometric analyses were performed using MS Windows (version 2007) operated MS Excel (version 2007) and Statistical Analysis System software version 9.2 (SAS Institute Inc., 2009). The main objective of the statistical analysis was to know the significance or otherwise of the variation exhibited by a character, between the individuals of a sample population and also between the various populations.

i. Coefficient of Variation (CV)

To evaluate the stability and consistency of a character and to assess the utility of a character for its diagnostic value the coefficient of variation analyses were done for each character.

ii. Principal Component Analysis (PCA)

This statistical procedure was used to reduce the number of variables by projecting the original measured variables into a new set of variables that has some inherent statistical properties. This analysis is thus used to investigate morphological
variations and thereby exploring differences among the groups by effectively reducing the dimension of the problem. The total number of principal components that could be generated is the total number of measurements considered. Since the number of measurement is very high, using the principal component analysis, it is possible to select first few principal components, which have large variations and could explain the differences in the populations.

Principal component analysis was performed on the correlation matrix for the 40 variables (2 meristic and 38 ratio characters) to determine the effects of size and shape on the distribution of scores along the first four principal component axes (Sokal and Rohlf, 1995) and observe their distribution without constraints of prior assignment to particular populations. Thereafter cluster analysis was performed following the AVERAGE method and a hierarchical cluster was constructed.

5.3 Result

5.3.1 Coefficient of variation (CV) analyses

A total of forty quantitative characters crucial in defining *D. rapae* at all its taxonomic levels were selected for morphometric studies on the eleven populations of *D. rapae* which were (Table 4.1 and Fig. 5.1-5.8). The quantitative characters were of two kinds: meristic and ratios (Table 5.2). With an objective of getting information on the validity of the morphometric characters utilized in the present study, statistical analyses were undertaken for evaluating the extent of variation within or among the populations. Table 4.3 represents all 40 quantitative characters with descriptive statistics parameters and summary statistics of logarithmic transformed data for all the populations separately. Analysis of coefficient of variation revealed that the characters are not equally consistent throughout all the eleven population. For example, LFL/LF1 (Length of last flagellomere/length of flagellomere I) was found with highest and lowest CV values of 18.23 and 7.42 in the population of IND MUS and PANT CAB, respectively. Characters showing CV values higher than 10 within the populations were inconsistent and therefore to be used with care. For example, tentorial index (OT/IT: Tenterio-ocular line/inter tentorial line) is one commonly used character in identification of different Aphidiine species, but in our study the CV
value of OT/IT ranges from 8.61 (BAR CAB) to 29.31 (PANT CAB) suggesting careful use of this character in discriminating different populations of *D. rapae*.

On the other hand, the character showing more CV among different populations would be more helpful in discriminating different populations. CV values along with the summary statistics of all quantitative (meristic and ratio) characters of studied populations of *D. rapae* are represented in Table 5.1. Sixteen characters were found with CV values higher than 10 and hence could be considered as valuable characters for discriminating different populations. These sixteen characters as follows: LFL/LF1 (Length of last flagellomere/length of flagellomere I); LFL/WFL (Length of last flagellomere/width of last flagellomere); OT/IT (Tenterio-ocular line/inter tentorial line); AO/DAT (Antennal socket ocular line/distance between antennae); IOR/IOC (Intero orbital distance/intero ocellar distance); OOC/IOC (Oculo ocellar distance/intero ocellar distance); WM/GW (Mandible width at base/width of gena); LPN/LMN (Length of pronotum/length of mesonotum); LMC/LCT1 (Length of median longitudinal carina on propodeum/length of dorsal carina on first tergum); WCA/WMP (Width of central areola/width of mesopleuron); LFW/FWF (Length of fore wing/maximum width or fore wing); WSt/LSt (Width of stigma/length of stigma); DTR1/DTA (Distance between tip of r and Rs and base of R1/distance between tip of r and Rs and apex of wing); LOS/LT1 (Length of ovipositor sheath/length of tergite I); LOS/WOS (Length of ovipositor sheath/width of ovipositor sheath at base) and NLCP (Number of lateral carinae on tergite I). Among these sixteen characters IOR/IOC (Intero orbital distance/intero ocellar distance) is with lowest CV value of 10.32, hence is expected to have minor role in discriminating different populations and LPN/LMN (Length of pronotum/length of mesonotum) with highest CV value of 28.88, hence is expected to contribute significantly in differentiating the eleven populations.

**5.3.2 Principal Component Analysis (PCA)**

PCA was used to reduce the number of variables by projecting the original measured variables into a new set of variables that has some inherent statistical properties. This analysis is thus used to investigate morphological variations and thereby exploring differences among the groups by effectively reducing the dimension of the problem.
The first two principal components contributed to 37% of the total variance (PC 1 = 20% and PC 2 = 18%). The third, fourth and fifth components contributed 14%, 12% and 10%, respectively. Cumulatively, 73% of the variability was contributed by first five principal components. Eigen values for the first, second, third, fourth and fifth principal components were 7.83, 7.08, 5.42, 4.62 and 4.12, respectively. Ten characters which were found having higher contribution in total variance of first principal component are as follows: LF1/LF2 (Length of flagellomere I/length of flagellomere II); LFL/LF1 (Length of last flagellomere/length of flagellomere I); LF1/WF1 (Length of flagellomere I/width of flagellomere I); LFL/WFL (Length of last flagellomere/width of last flagellomere); WMP/LMES (Width of mesopleuron/length of mesosoma); WMP/LHT (Width of mesopleuron/length of hind tibia); LMB/WFW (Length of basal part of M/maximum width or fore wing); DTR1/DTA (Distance between tip of r and Rs and base or R1/maximum width or fore wing); DSrRS/LSt (Distance between base of stigma and r and Rs/length of stigma); LMCu/L1CuA (Length of M+Cu/length of 1CuA) (Table 5.2). Among these ten characters three characters, namely, LFL/LF1, LFL/WFL and DTR1/DTA was recorded with negative value. In second principal component eight characters contributed heavily, four with positive value, namely, TW/GW (Width of temple/width of gena); LED/TED (Longitudinal eye diameter/transverse eye diameter); LFW/WFW (Length of fore wing/maximum width or fore wing); LR1/LrRS (Length of metacarp (R1)/length of r and Rs) and four with negative value, namely, NAS (Number of antennal segments); AO/DAT (Antennal socket ocular line/distance between antennae); WPR/LMN (Width of propodeum/length of mesonotum) and LT1/LA (Length of tergite I/length of abdomen) (Table 5.2). In third principal component following ten characters have higher contribution namely, LPN/LMN (Length of pronotum/length of mesonotum); LMN/WMT (Length of mesonotum/width of mesonotum at tegulae); WMP/LHT (Width of mesopleuron/length of hind tibia); LFW/WFW (Length of fore wing/maximum width or fore wing); LCSC/LFW (Length of costa + subcosta/length of fore wing); LMB/WFW (Length of basal part of M/maximum width or fore wing); DTR1/WFW (Distance between tip of r and Rs and base or R1/maximum width or fore wing); LMCu/L1CuA (Length of M+Cu/length of 1CuA); LT1/LA (Length of tergite I/length of abdomen) and LOS/LT1 (Length of ovipositor sheath/length of tergite I). Among these ten characters four contributed negatively namely, LPN/LMN, WMP/LHT,
LCSC/LFW and LOS/LT1 (Table 5.2). In fourth principal component again ten characters were recorded with higher contribution namely, NAS (Number of antennal segments); OT/IT (Tenterio-ocular line/inter tentorial line); LH/WH (Length of head/width of head); OOC/IoC (Oculo ocellar distance/intero ocellar distance); LMN/WMT (Length of mesonotum/width of mesonotum at tegulae); WCA/WMP (Width of central areola/width of mesopleuron); LHC/LHF (Length of hind coxa/length of hind femur); DTR1/WFR (Distance between tip of r and Rs and apex of wing/length of fore wing); DTR1/DTA ((Distance between tip of r and Rs and apex of wing/length of fore wing) and LOS/WOS (Length of ovipositor sheath/width of ovipositor sheath at base). Among these ten characters, OOC/IoC, WCA/WMP, LHC/LHF and LOS/WOS were recorded with negative values (Table 5.2). Twelve characters were found contributing heavily in the fifth principal component namely OT/IT (Tenterio-ocular line/inter tentorial line); IOR/IoC (Intero orbital distance/intero ocellar distance); WM/GW (Mandible width at base/width of gena); LED/TED (Longitudinal eye diameter/transverse eye diameter); WPR/LMN (Width of propodeum/length of mesonotum); LMC/LCT1 (Length of median longitudinal carina on propodeum/length of dorsal carina on first tergum); WCA/WMP (Width of central areola/width of mesopleuron); LHB/LHT (Length of hind basitarsus/length of hind tibia); LCSC/LFW (Length of costa + subcosta/length of fore wing); DTA/LFW (Distance between tip of r and Rs and apex of wing/length of fore wing); LOS/WOS (Length of ovipositor sheath/width of ovipositor sheath at base) and NLCT (Number of lateral carinae on tergite I). Five of these character among them viz., OT/IT, IOR/IoC, LMC/LCT1, LCSC/LFW and DTA/LFW were found to have negative contribution (Table 5.2).

Hierarchical cluster between the eleven *D. rapae* populations used in the study (using AVERAGE clustering method) has been sown in Fig. 5.1. Two groups were formed: Group 1 dominated by population from planes and Group 2 dominated by population from regions of higher altitude. ALI CAB and DEL CAB were placed as the most closely related populations in the first group, with PANT MUS joining as a sister group to the two. Again ALI CAB, DEL CAB and PANT MUS are more closely related to IND MUS, with DER MUS serving as the first branch to the first group. In the second group NAI MUS and PANT CAB are placed as the most closely related populations, with ALM MUS joined as a sister group to the two. Again ALM
CAB and BAR CAB were more closely related and joining as a sister group to NAI MUS, PANT CAB and ALM MUS sub group, with DEL MUS serving as the first branch to the second group.

5.4 DISCUSSION

5.4.1 Variability of characters

Sixteen characters viz. LFL/LF1, LFL/WFL, OT/IT, AO/DAT, IOR/IOC OOC/IOC, WM/GW, LPN/LMN, LMC/LCT1, WCA/WMP, LFW/WFW, WSt/LSt, DTR1/DTA, LOS/LT1, LOS/WOS and NLCP (Table 5.1) were found with CV values higher than 10 and hence can be considered as valuable character for discriminating different populations. Among these sixteen characters seven were head characters, viz. LFL/LF1, LFL/WFL, OT/IT, AO/DAT, IOR/IOC OOC/IOC and WM/GW; three were thoracic characters, viz. LPN/LMN, LMC/LCT1 and WCA/WMP; three were fore wing characters, viz. LFW/WFW, WSt/LSt and DTR1/DTA and rest of the three were abdominal characters, viz. LOS/LT1, LOS/WOS and NLCP. All of these characters exhibit higher contribution towards the overall morphological variability, especially the head characters towards differentiation of the eleven *D. rapae* populations. Observations during the present studies are in agreement with those of Woolley *et al.* (1994).

5.4.2 Principal components and cluster analyses

Large specimens tend to have larger dimensions (in the absence of allometry) and have a great deal of variance associated with overall size (Woolley *et al.*, 1994). To minimize this effect, measurements were expressed as ratios (Sokal and Rohlf, 1995) to equalize the standard deviations across differently-sized variables and ensure multivariate normality of the data (Woolley and Browning, 1987). Separations along the first principal axes are usually associated with overall size, while those along the second principal axes are associated with shape (Jolicoeur and Mosimann, 1960).

Table 5.2 shows the weights of the first principal component within small range, an indication of the role played by overall size in separation of the populations. Mixed magnitudes and large range values are situations where separations along the first and second principal axes are more than the simplistic association with overall size and shape (Bookstein *et al.*, 1985; Rohlf and Bookstein, 1987, 1990). The major
contributing variables in first principal component were LF1/LF2 (Length of flagellomere I/length of flagellomere II); LFL/LF1 (Length of last flagellomere/length of flagellomere I); LF1/WF1 (Length of flagellomere I/width of flagellomere I); LFL/WFL (Length of last flagellomere/width of last flagellomere); WMP/LMES (Width of mesopleuron/length of mesosoma); WMP/LHT (Width of mesopleuron/length of hind tibia); LMB/WFW (Length of basal part of M/maximum width or fore wing); DTR1/DTA (Distance between tip of r and Rs and base or R1/maximum width or fore wing); DSrRS/LSt (Distance between base of stigma and r and Rs/length of stigma); LMCu/L1CuA (Length of M+C u/length of 1Cua) with LF1/WF1, WMP/LMES and LMCu/L1CuA having highest positive value of 0.27 and LFL/WFL having highest negative value of -0.31. All of these findings reflect the importance of relative size and shape in differentiating populations and the present findings are similar to the observation reported in Psyttalia spp. (Hymenoptera: Braconidae) by Billah et al. (2008).

Another interesting finding during the present studies is that the relative dominance of characters associated with the wing in first principal component, represented by the fact that four out of the ten characters having greater contribution in first principal component were wing characters. These findings again emphasize the fact that wing characters have got tremendous importance in differentiating different intra specific population that has been already established for several Aphidiinae species (Stary 1961, 1970, 1988).

Although hierarchical cluster between the eleven D. rapae populations used in the study (using AVERAGE clustering method) has formed two groups: Group 1 dominated by population from planes and Group 2 dominated by population from higher altitude, the analysis failed to cluster the nine populations in distinct groups of plane and high altitude populations. Effect of host plants viz., cabbage and mustard also appear to play a minimal role in clustering of the populations. Presence of DER MUS (a high altitude population; altitude 652 m), in the first group dominated by population from planes as well as PANT CAB (a north Indian plain zone population; altitude 234 m) and DEL MUS (a plain population; altitude 223 m) in the second group dominated by population from higher altitude cannot be satisfactorily explained. However a few recent studies on phylogeography and host-related fitness trade-offs of D. rapae based on molecular study concluded that, this species being
cosmopolitan and polyphagous ongoing gene flow appears to prevent the formation of host or geographical races and it appeared that gene flow was sufficient to prevent formation of separate lineages (Baer et al., 2004; Antolin et al., 2006). Keeping these evidences in mind, in our case it can be hypothesized that being widely distributed in different parts of India, with wide host range, formation of host race as well as geographical race in *D. rapae* appears to be a remote possibility. Nevertheless some variability may be observed between the populations of plains and hills.

Thus it can be concluded from the present studies that further comparison of different populations of *D. rapae* should be focused on detailed search involving more heterogeneous populations from different parts of India combining both morphometric and molecular methods. Practically, the study and characterization of different *D. rapae* population may help us to identify different populations with varied biological and ecological adaptability, which may improve the mustard aphid management incorporating *D. rapae*, promoting sustainable crop protection.

**NOTE:** During the present studies differentiation of the populations of *D. rapae* at the molecular level was also attempted but as the sequences for the ITS region were not received due to paucity of time they could not be included.
**Table 5.1** Summary statistics of quantitative (meristic and ratio) characters of studied populations of *D. rapae*

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x- Mean, SD- standard deviation, SV- sample variance, CV- coefficient of variation of raw data, x’ – mean, SD’ – standard deviation, SV’ – sample variance, CV’ - coefficient of variation of logarithmic transformed data.
Table 5.2: Eigen values and weights of the first five principal components, based on correlation matrix of the eleven *D. rapae* populations from different parts of India.

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Seasonal occurrence, distribution and sampling indices for *Lipaphis erysimi* (Hemiptera: Aphididae) and its parasitoids (Hymenoptera: Braconidae: Aphidiinae) on mustard

ABSTRACT

Field studies to assess the seasonal occurrence and the spatial distribution of parasitoids of *Lipaphis erysimi* (Sulzer) on mustard, viz., *Aphidius colemani* Viereck, *Diaeretiella rapae* (M’Intosh) were conducted at the research farm of Indian Agricultural Research Institute, New Delhi, India (28°4’N, 77°09’E and 223.06m above mean sea level) during two consecutive *rabi* seasons of 2009-10 and 2010-11. *Brassica juncea* variety Pusa bold was sown in 5 plots sized 8m×10m. Sowing was done on 17th November and 22nd November during 2009 and 2010 respectively. All the recommended agronomic practices were followed to raise a good crop. No insecticidal spray was given to avoid mortality of natural enemies. Sampling started with the first colonization of mustard plants by aphids (17th January and 23rd January in 2010 and 2011 respectively) and continued until the last harvest of plants (21st March and 27th March in 2010 and 2011 respectively) at weekly intervals and at each sampling, five plants were chosen randomly from each plot i.e. samples were collected from a total of 25 plants. Each sampling unit comprised of three subunits, viz., terminal 10cm twig, one leaf each from the middle and lower region of the plant. The distribution of the species found was also recorded and discussed. Generally, high *L. erysimi* densities were found in second half of February during both years. The mummification rate also showed an increasing trend late in the season (first half of March). In 2010, the percentage of mummification reached 53.18 per cent at the end of the season whereas in 2011 it was higher and reached 65.5 per cent. The density of *L. erysimi* individuals were higher on the terminal 10 cm shoot of the plants compared to those collected from the leaves in the middle and lower part in both years but without significant difference. In contrast, the numbers of mummified *L. erysimi*
individuals were higher on leaves collected from the middle part of the plants than on those from the terminal 10 cm shoot and lower part in both years. The relative abundance of *A. colemani* and *D. rapae* differed between the two years. Seasonal abundance, hyperparasitic potential and distribution of *Pachyneuron aphidis* (Bouche) (Pteromalidae: Hymenoptera), a hyperparasitoid of *D. rapae*, on mustard was also discussed.

**Key words.** Aphidoidea, *Lipaphis erysimi*, Aphidiinae, *Aphidius colemani*, *Diaeretiella rapae*, mustard, seasonal occurrence, sampling indices

### 6.1 INTRODUCTION

Oilseed *Brassicas* are among the major oilseed crops cultivated in India and around the world. India produces about 11.3 per cent of the world’s rapeseed-mustard (Damodaram and Hegde, 2002). Mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae) is the main pest infesting mustard in several areas in India and its infestation reduces directly both yield and quality of mustard and causes up to 83 per cent losses (Mandal *et al*., 1994). Resistance to certain insecticides has also been recorded against this species from some parts of the world (Wei *et al*., 1988). The economic injury level of this pest is 2.04 aphids/plants (Singh and Malik, 1989) and increases in population beyond 9.45 aphids per plant, reduces the seed yield by 59.3 per cent. Considerable work has been done to assess the role of *D. rapae* in suppressing *L. erysimi* in mustard. *D. rapae* is described as one of the most important factor for natural control of mustard aphid (Akhtar *et al*., 2010; Dhiman, 2007; Dogra *et al*., 2003; Pike *et al*., 1999). On the other hand, *D. rapae* females were found to be attracted more by crucifer plants compared to other plant types (Sheehan and Shelton, 1989; Vaughn *et al*., 1996). Furthermore, *D. rapae* and *L. erysimi* prefer the same host plant possibly because both of them respond positively to the volatile compounds produced by these plants (Bundemerg, 1990). It was also reported that honeydew emitted by aphids act as kairomones for its natural enemies (Brown *et al*., 1970; Dicke and Sabelis, 1988). The aforementioned studies provide useful information about the seasonal activity of aphidophagous species especially, *D. rapae*; however, in most of the above studies the capacity of these species in suppressing aphid populations and their practical utilization was not assessed under field conditions. The
high density of beneficials that are often reported to occur in mustard fields clearly suggest that an integration of natural enemies for aphid management is feasible, and these agents can play a key role in an Integrated Pest Management based strategy.

Kavallieratos et al., (2005) studied seasonal occurrence, distribution and sampling indices for *Myzus persicae* and its aphidophagous parasitoids on tobacco in western Greece. Although, Dhiman (2007) observed maximum number of mummies on the ventral side of the leaves and inflorescence, still information is inadequate on the distribution of both *L. erysimi* and its parasitoids on mustard. Estimate of pest and parasitoid densities along with their distribution on plants is essential for identification of a suitable sampling unit and incorporation of the natural enemy threshold which might in turn lead to a rational aphid management in mustard. If locally developed, aphid populations are detected, the release of parasitoids in those areas may be preferred before the extensive insecticidal applications.

Often it has been reported that the aphid hyperparasitoids reduce the parasitic potential of aphidines in several crops (Dhiman, 2006). Although Pandey et al., 1985 reported *Pachyneuron aphidis* (Bouche) (Pteromalidae: Hymenoptera) as a hyperparasitoid of *D. rapae*, for the first time from India, little is known about their hyperparasitic potential, seasonal abundance and distribution on mustard plant. In our study, we examined the seasonal occurrence and the distribution of aphids, parasitoids and hyperparasitoids on mustard, over a two-year period.

6.2 MATERIAL AND METHODS

6.2.1 Experimental site

The study was conducted at the research farm of Indian Agricultural Research Institute, New Delhi, India (28°4’N, 77°09’E and 223.06m above mean sea level) during two consecutive *rabi* seasons viz., 2009-10 and 2010-11. *Brassica juncea* variety Pusa bold was sown in 5 plots sized 8m×10m. Sowing was done on 17th November and 22nd November during 2009 and 2010 respectively. All the recommended agronomic practices were followed to raise a good crop. No insecticidal spray was given to avoid mortality of natural enemies.
6.2.2 Sampling

Sampling during the previous years had indicated a heavy parasitisation of *L. erysimi*. Sampling was from the first appearance of aphids on mustard plants which was 17th January and 23rd January during 2010 and 2011 respectively and continued till the harvest of the crop on 21st March and 27th March during 2010 and 2011 respectively. Sampling was done at weekly intervals and at each sampling five plants were chosen randomly from each plot i.e. samples were collected from a total of 25 plants. Each sampling unit comprised of three subunits, *viz.*, terminal 10cm twig, one leaf each from the middle and lower region of the plant.

6.2.3 Processing and preservation of samples

Each plant part was enclosed separately in a plastic bag and then detached from the stem of the plant with a scissors. The bags were brought to the laboratory and aphids were identified to species. Mummies were placed separately in small plastic vials, covered with muslin cloth, tied with rubber band, and labelled with details of collection date and the serial number of the terminal 10cm shoot and leaves. Next, the plastic vials were kept under laboratory conditions of 26±5°C, 65±5 per cent RH and 16L: 8D until emergence of the adult parasitoid/hyperparasitoid.

6.2.4 Data analysis

The mean total number of aphids, percentage of mummified aphids to the total number of aphids (Tomanovic *et al.*, 1996; Kavallieratos *et al.*, 2002a, b, 2004), the percentage of hyperparasitized mummies to the total number of mummies and percentage of *D. rapae*, *A. colemani* and *P. aphidis* to the total number of mummies per terminal 10cm shoot and middle and lower leaf were calculated per sampling date.

Analysis of variance was done after transformation of the data $y_{trans} = \sqrt{(y + 0.5)}$ to normalise variances and standardise means using the statistical package SPSS 16.0. One way ANOVA was used to test the significance of differences in the total number of: (a) *L. erysimi* individuals present among the three sampling subunits, *viz.*, terminal 10cm shoot, middle and lower leaf of the plants; (b) species of aphidine parasitoids (*D. rapae* and *A. colemani*) which emerged from each sampling sub unit; (c) *D. rapae* individuals which emerged from each sampling sub unit; (d) *A. colemani* individuals which emerged from each sampling sub unit; (e) hyperparasitoids which
emerged from each sampling sub unit during the entire period of the study for each year. Means were finally compared with the Tukey-Kramer (HSD) test (at $P = 0.05$).

### 6.3 RESULT

#### 6.3.1 Distribution of $L. erysimi$

$L. erysimi$ was the only aphid species infesting mustard at IARI, New Delhi during the present studies. Average population of $L. erysimi$ per plant i.e., combined population on the terminal 10 cm shoot, middle and lower leaf increased rapidly after the 7th standard during 2010 and after 6th standard week during 2011. Peak population was reached during 9th standard week in 2010 (608 ± 49.2 individuals per sampling unit) (Fig. 6.1A) and 10th standard week in 2011 (487.1 ± 35.2 individuals per sampling unit) (Fig. 6.1B). High aphid densities were observed during the 2010 growing season from mid January which increased rapidly but comparatively lower aphid densities were observed, except during the first half of March which decreased rapidly after 12th standard week (Fig 6.1A).

Observations on the aphid population densities on the various plant parts indicated a similar pattern on the terminal 10 cm shoot [peak on 9th standard week, 348.6 ± 31.4 individuals per terminal 10 cm shoot in 2010 (Fig. 6.2A); peak on 10th standard week, 281.8 ± 27 individuals per terminal 10 cm shoot in 2011 (Fig.6.2B)]; middle leaf [peak on 9th standard week, 170.8 ± 15.6 individuals per leaf in 2010 (Fig. 6.3A) peak on 10th standard week, 145.6 ± 12 individuals per leaf in 2011 (Fig. 6.3B)] and lower leaf of the plant [peak on 9th standard week, 88.5 ± 10.3 individuals per leaf in 2010 (Fig. 6.4A); peak on 10th standard week, 59.8 ± 7.9 individuals per leaf in 2011 (Fig. 6.4B)]. The numbers of $L. erysimi$ individuals were higher on the terminal 10 cm shoot ($\bar{x} = 109.0$ in 2010; $\bar{x} = 127.8$ in 2011) of the plants compared to those collected from the leaves in the middle ($\bar{x} = 54.3$ in 2010; $\bar{x} = 53.8$ in 2011) and lower ($\bar{x} = 29.05$ in 2010; $\bar{x} = 23.0$ in 2011) parts during both years (Table 5.10, 5.11) but without significant difference ($F = 2.35; df = 2, 27; P = 0.114$) in 2010. In 2011 the numbers of $L. erysimi$ individuals were significantly higher on the terminal 10 cm shoot of the plants compared to lower leaves ($F = 4.68; df = 2, 27; P = 0.018$).
6.3.2 Distribution of mummified aphids

Mummified aphids were observed from fourth sampling onwards i.e. from the 6th standard week in 2010 and third sampling i.e. 6th standard week during 2011 (Fig. 6.12A, 6.12B). Average number of mummified aphids per plant (combined population of all the three subunits i.e. top terminal 10 cm shoot, middle and lower leaf) was maximum during the 11th standard week (62.2 ± 4.6 mummies per sampling unit) and 12th standard week (79.8 ± 4.7 mummies per sampling unit) during 2010 and 2011, respectively (Fig. 6.5A, 6.5B). During both years more mummies were found in first half of March, on the leaf collected from middle half of the plants (Fig. 6.7A, 6.7B). Mummified aphid population densities followed a similar pattern on the terminal 10 cm shoot [peak on 11th standard week, 26.1 ± 2.3 mummies per terminal 10 cm shoot in 2010 (Fig. 6.6A) while during 2011 peak population was reached during the 12th standard week (29.4 ± 1.8 mummies per terminal 10 cm shoot (Fig. 6.6B)], on the middle leaf [peak on 11th standard week, 30.2 ± 2.4 mummies per middle leaf in 2010 (Fig. 6.7A); peak on 12th standard week, 37.3 ± 2.7 mummies per middle leaf in 2011 (Fig. 6.7B)] and on the lower leaf of the plant [peak on 11th standard week, 5.8 ± 1.2 mummies per lower leaf in 2010 (Fig. 6.8A); peak on 12th standard week, 13.1 ± 1.4 mummies per lower leaf in 2011 (Fig. 6.8B)]. Numbers of mummified *L. erysimi* individuals were higher on leaves collected from the middle (\(\bar{x} = 6.5\) in 2010; \(\bar{x} = 7.6\) in 2011) part of the plants than on terminal 10 cm shoot (\(\bar{x} = 5.0\) in 2010; \(\bar{x} = 6.9\) in 2011) and lower (\(\bar{x} = 1.4\) in 2010; \(\bar{x} = 2.2\) in 2011), leaves but without significant differences \([F = 1.05; df = 2, 27; P = 0.36\) in 2010; \(F = 1.33; df = 2, 27; P = 0.28\) in 2011]. The mummification rate showed a specific increasing trend late in the season (first half of March). In 2010, the percentage of mummification reached 53.18 per cent at the end of the season (Fig. 6.1A) whereas in 2011 it was higher and reached 65.5 per cent (Fig. 6.1B).

6.3.3 Relative abundance of aphidines

*L. erysimi* was parasitized by *A. colemani* and *D. rapae* during both the years (Fig. 6.12A, 6.12B). *D. rapae* was observed 6th and 7th standard week onwards during 2010 and 2011, respectively (Fig. 6.12A, 6.12B) while *A. colemani* was seen from 8th and 6th standard week in 2010 and 2011, respectively (Fig. 6.12A, 6.12B). Although parasitoid was first recorded from terminal 10 cm shoot, most of them were found from middle leaf during the first fortnight of March. *A. colemani* was more abundant...
on terminal 10 cm shoot [peak on 12th standard week, 3.12 ± 0.45 individuals per terminal 10 cm shoot in 2010 (Fig. 6.9A, 6.13A); peak on 13th standard week, 15.4 ± 1.3 individuals per terminal 10 cm shoot in 2011 (Fig. 6.9B, 6.13B)], as compared to middle leaf [peak on 12th standard week, 2.7 ± 0.5 individuals per leaf in 2010 (Fig. 6.10A, 6.14A) peak on 13th standard week, 9.3 ± 1.3 individuals per leaf in 2011 (Fig. 6.10B, 6.14B)] and lower leaf of the plant [peak on 12th standard week, 0.08 ± 0.05 individuals per leaf in 2010 (Fig. 6.11A, 6.15A); peak on 13th standard week, 2.5 ± 0.55 individuals per leaf in 2011 (Fig. 6.11B, 6.15B)] but without significant differences [F = 2.91; df = 2, 27; P = 0.07 in 2010; F = 3.1; df = 2, 27; P = 0.61 in 2011]. 

D. rapae was more abundant on leaves collected from middle part of the plant [peak on 12th standard week, 25.7 ± 2.2 individuals per middle leaf in 2010 (Fig. 6.10A, 6.14A)]; peak on 13th standard week, 26.1 ± 1.9 individuals per middle leaf in 2011 (Fig. 6.10B, 6.14B), as compared to terminal 10 cm shoot [peak on 12th standard week, 22.2 ± 2.2 individuals per leaf in 2010 (Fig. 6.9A, 6.13A) peak on 13th standard week, 12.6 ± 1.6 individuals per leaf in 2011 (Fig. 6.9B, 6.13B) and lower leaf of the plant [peak on 12th standard week, 5.6 ± 1.1 individuals per leaf in 2010 (Fig. 6.11A, 6.15A); peak on 13th standard week, 10.2 ± 1.2 individuals per leaf in 2011 (Fig. 6.11B, 6.15B)] but without significant differences [F = 1.02; df = 2, 27; P = 0.37 in 2010; F = 1.2; df = 2, 27; P = 0.32 in 2011].

### 6.3.4 Distribution of aphidines

Although average number of D. rapae individuals (x̄ = 11.06 and x̄ = 9.97 in 2010 and 2011 respectively) collected from mustard plants (combined population on the terminal 10 cm shoot, middle and lower leaf) was higher than A. colemani (x̄ = 1.22 and x̄ = 6.05 in 2010 and 2011 respectively), ANOVA showed no significant differences in numbers between these two aphidines that parasitized L. erysimi during both years (F = 3.07; df = 1, 18; P = 0.096 in 2010; F = 0.236; df = 1, 18; P = 0.633 in 2011).

Average number of D. rapae individuals (x = 3.94) collected from terminal 10 cm shoot was higher than A. colemani (x̄ = 0.86) in 2010 (Fig. 6.9A), but without significant difference (F = 1.45; df = 1, 18; P = 0.24). In 2011, A. colemani (x̄ = 0.86) dominated over D. rapae [x̄ = 3.94) (Fig. 6.9B)] on terminal 10 cm shoot but without significant difference (F = 0.327; df = 1, 18; P = 0.575).
Average number of *D. rapae* individuals (\(\bar{x} = 5.78\) and \(\bar{x} = 5.52\) in 2010 and 2011 respectively) per leaf collected from middle portion of the plant was higher than *A. colemani* (\(\bar{x} = 0.35\) and \(\bar{x} = 1.76\) in 2010 and 2011 respectively) (Fig. 6.10 A, 6.10 B) with significant difference (\(F = 4.698; df = 1, 18; P = 0.044\)) in 2010, but without significant difference [\(F = 1.692; df = 1, 18; P = 0.210\)] in 2011.

ANOVA showed no significant differences in numbers between average number of *D. rapae* (\(\bar{x} = 1.35\) and \(\bar{x} = 1.72\) in 2010 and 2011 respectively) and *A. colemani* [(\(\bar{x} = 0.01\) and \(\bar{x} = 0.41\) in 2010 and 2011 respectively) (Fig. 6.11A, 6.11B)] per leaf collected from lower part of mustard plants in both of the years [\(F = 3.591; df = 1, 18; P = 0.074\) in 2010; \(F = 1.114; df = 1, 18; P = 0.305\) in 2011].

**6.3.5 Distribution of hyperparasitoids**

Mummies hyperparasitized by *P.aphidis* were recorded from the eighth sampling i.e. the 10\(^{th}\) standard week in 2010 and 6\(^{th}\) standard week in 2011. Percentage hyperparasitization per plant (combined hyperparasitization on the terminal 10 cm shoot, middle and lower leaf) peaked on 12\(^{th}\) standard week (30.51 per cent hyperparasitized mummies per sampling unit) and 13\(^{th}\) standard week (18.84 per cent hyperparasitized mummies per sampling unit) in 2010 and 2011, respectively (Fig. 6.5A, 6.5B). During both years higher percentage of hyperparasitized mummies were found on the last sampling date, on the leaf collected from middle half of the plants (Fig. 6.7A, 6.7B). Percent hyperparasitized mummies followed a similar pattern on the terminal 10 cm shoot [peak on 12\(^{th}\) standard week, 26.49 per cent hyperparasitized mummies per terminal 10 cm shoot in 2010 (Fig. 6.6A); peak on 13\(^{th}\) standard week, 15.94 per cent hyperparasitized mummies per terminal 10 cm shoot in 2011 (Fig. 6.6B)], on the middle leaf [peak on 12\(^{th}\) standard week, 34.78 per cent per cent hyperparasitized mummies per middle leaf in 2010 (Fig. 6.7A); peak on 13\(^{th}\) standard week, 22.22 per cent hyperparasitized mummies per middle leaf in 2011(Fig. 6.7B)] and on the lower leaf of the plant [peak on 12\(^{th}\) standard week, 6.67 per cent hyperparasitized mummies per lower leaf in 2010 (Fig. 6.8A); peak on 13\(^{th}\) standard week, 5.88 per cent hyperparasitized mummies per lower leaf in 2011(Fig. 6.8B)]. Average number of hyperparasitized mummies by *P. aphidis* was higher on the leaf collected from middle portion of the mustard plant (\(\bar{x} = 0.34\) and \(\bar{x} = 0.36\) in 2010 and 2011 respectively) than on the terminal 10 cm shoot (\(\bar{x} = 0.17\) and \(\bar{x} = 0.28\) in 2010 and 2011 respectively) and the leaf collected from lower portion of the plant.
(\bar{x} = 0.02 and \bar{x} = 0.04 in 2010 and 2011 respectively) in both years, but without significant difference (\( F = 1.313; df = 2, 27; P = 0.305 \) in 2010; \( F = 0.997; df = 2, 27; P = 0.382 \) in 1997).

### 6.3.6 Correlation with weather parameters

The data recorded on the total aphid population, total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined and also total per cent hyperparasitisation due to *P. aphidis* were correlated with data of various abiotic parameters, viz., maximum and minimum temperature, mean temperature, rainfall, average wind speed, average relative humidity, sunshine hours and evaporation during both the years (Table 6.1). Perusal of the data showed that maximum, minimum and mean temperature showed significant positive correlation with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined and also total per cent hyperparasitisation due to *P. aphidis* during both the years of study except with total per cent parasitisation due to *A. colemani* alone during 2010. Although rainfall showed a positive correlation with the total aphid population and a negative correlation with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined and also total per cent hyperparasitisation due to *P. aphidis* but none of the correlations were significant. Average wind speed affected significantly only the total aphid population during 2010. Relative humidity showed negative correlation with all the parameters during 2010 but the correlation was significant only with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined. However relative humidity showed significant positive correlation with the aphid population only during 2011. Bright sunshine showed a significant positive correlation with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined during 2010 and a significant positive correlation with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined and also total per cent hyperparasitisation due to *P. aphidis* during 2011. However its correlation with the total aphid population was not significant during both the years. Evaporation in mm showed a very strong positive correlation with total per cent parasitisation due to *D. rapae, A. colemani* individually and also combined and also total per cent hyperparasitisation due to *P. aphidis* during both 2010 and 2011.
6.4 DISCUSSION

6.4.1 Seasonal abundance of aphids

During both the years, i.e., crop season of 2009-10 and 2010-11 the aphid count per plant increased rapidly since their first appearance in the field. The rapid and sudden reduction in aphid populations on terminal 10 cm shoot observed during the 9th standard week in 2011 could probably be attributed to a sharp rainfall (7 mm) received on 26th February, a day just before the sampling date (Fig 6.2B). Aphids were found to have been dislodged from the shoot and could be seen collected on the base of the plant. Again a sharp decline in aphid count per plant was observed after the 10th standard week during 2010 and 12th standard week during 2011. This could have been due to the relatively high mean maximum temperature of the preceding week which was 31.3°C and 30.9°C in 2010 and 2011 respectively (Fig. 6.1A, 6.1B). Due to this sudden increase in temperature and other unfavourable conditions like crowding and poor host plant quality, alate adults of aphids were produced and migration followed leading to the sudden crash in aphid population (Hardie and Lees, 1985; Chapman, 1998). During both the years highest per cent mummification due to parasitism [53.1 per cent and 65.5 per cent in 2010 and 2011, respectively (Fig. 6.1A, 6.1B)] was observed on the last date of sampling, when mean aphid densities per plant was very low [12.9 ± 2.2 and 22.0 ± 2.2 (Fig. 6.1A, 6.1B)] Hence, the results of the present study may suggest that parasitoids did not reduce aphid densities in either of the years tested, given that the aphid presence was high for most of the sampling period. This could be attributed to their delayed action since, in agreement with Stary (1988), the first mummies generally appeared after the increase of aphid population density to high levels. Our study also revealed the same trend as there was a three weeks lag period in 2010 and two weeks lag period in 2011 between the colonization by aphids of the first plant and occurrence of the first mummified individuals (Fig. 6.12A, 6.12B). However, the decreasing trend in aphid population which coincided with the increasing per cent mummification during the first half of March during both years suggests that parasitoids do regulate the aphid population partially, at least, at the latter half of the mustard growing season. The higher percentage of mummification observed on the leaves collected from middle part of the mustard plant during both years could be attributed to the production of honeydew on the more populated terminal parts of the plant which restricts oviposition by the parasitoids.
through their immobilization (Stary, 1970). Furthermore, aphids in large colonies increase at such a high rate that the parasitoids can attack only a small percentage of them (Stary, 1970).

6.4.2 Species of parasitoids

Our study indicated that *A. colemani* and *D. rapae* were the principal parasitoid species of *L. erysimi* on mustard at Delhi. *D. rapae* dominated over *A. colemani* on almost all three sampling sub units (terminal 10 cm shoot, middle leaf and lower leaf) during both the years, except on terminal 10 cm shoot during 2011, wherein *A. colemani* was found acting as the principal parasitoid of *L. erysimi* (Fig. 6.9, 6.10, 6.11). In 2010 there was significantly higher parasitism by *D. rapae* than that by *A. colemani*. But in 2011, although *D. rapae* was higher in abundance compared to *A. colemani*, statistical analyses revealed no significant difference between the abundance of the two parasitoid species.

6.4.3 Distribution within plants

During our study higher number of mummified aphids were found on the leaves collected from the middle part of the plant as compared to the either terminal 10 cm shoot or leaves collected from lower half of the plant (Fig. 6.6, 6.7, 6.8). During both the years *D. rapae* was always found to be more abundant on middle leaves as compared to terminal 10 cm shoot and lower leaves whereas, *A. colemani* preferred to remain on terminal 10 cm shoot in greater number than in other parts of the plant. Within plant distribution of *A. colemani* mummies on mustard is similar with the host behavior of another species of *Aphidius*, viz., *A. nigripes* Ashmead, a parasitoid of *Macrosiphum euphorbiae* (Thomas) on *Solanum tuberosum* L., where mummies were found particularly on the apical stratum of the plant. Brodeur and McNeil (1991, 1992) offered three possible explanations for the above in the apical parts of the potato plants: (a) parasitoids mummifying far from the aphid colony would be in sites with less honeydew and thus could avoid the detection by some predators, such as coccinellids as honeydew acts as a kairomone for several aphid predators (Ben Saad and Bishop, 1976); (b) the activity of the hyperparasitoids is restricted by the solar radiation. Further, hyperparasitoids being very tiny are subject to hydrothermic stresses when exposed to insolation for extended periods (Willmer and Unwin, 1981); (c) the solar radiation reduces the length of pupal development and
thus shortens the period the mummies are exposed to natural enemies. According to Brodeur and McNeil (1991) the *A. nigripes* mummies were evenly distributed over the terminal, median and bottom leaves of *Chenopodium album* L. plants. In contrast, in a recent study, Kavallieratos *et al.* (2005) showed that the *A. colemani* mummies were mainly found on the lower half of the tobacco plants during both years of the study period. In our study more number of *D. rapae* was recorded on the middle leaves compared to the terminal 10 cm shoot. Observations similar to the present results in mustard, the within plant distribution of *M. persicae* mummies on tobacco, have been reported by Lykouressis and Mentzos (1995). In view of the above three assumptions, during the present studies: (a) the quantity of honeydew was smaller on the lower leaves than the upper ones simply due to the presence of fewer aphids there. However, observations during the present studies indicated that mummies were found mostly within the aphid colony but some evidence was also there of movement by parasitized aphids which eventually fall on the upper side of the middle leaf. Surprisingly in most of such cases mummies were found to be parasitized by *A. colemani*. This is in confirmation with the views of Muller *et al.*, 1997 who had opined that many but not all aphid species leave the colony after parasitization (b) hyperparasitoids’ number were significantly higher on the leaf collected from middle half of the mustard plant where they were not exposed directly to the solar radiation. Thus, parasitoids suffered high levels of hyperparasitization on the middle leaves of the plants given that mummified aphids were more abundant on them compared to the terminal 10 cm shoot and lower leaves during both years of the study; (c) solar radiation seems to benefit *A. colemani* mummies but not *D. rapae* mummies since these “prefer” the shaded places of the tobacco plants. Similarly, mummies of *Aphidius rosae* (Haliday) attacking *Macrosiphum rosae* (L.) on *Rosa* sp., are found on the upper side of the leaves in spring whereas in summer, when the temperature and insolation are high, the majority of mummies occur on the lower side of the leaves (Fink, 1995). Generally, the plant architecture may influence interactions over several trophic levels (Brodeur and McNeil, 1991). Another possible explanation for the differential mummification on terminal 10 cm shoot middle and lower leaves is that parasitized aphids often drop from their feeding site and mummify on middle or lower leaves (Chow and Mackauer, 1999).
6.4.4 Spatio-temporal synchrony

One additional complication in assessing the spatial synchronization between aphids and parasitoids is the lack of spatio-temporal synchrony. In our study, although aggregated spatial patterns for both aphids and parasitoids may have been similar throughout the season, their spatial coincidence was dynamic, and this is the reason for the poor synchrony correlation. Apparently, parasitoids can reduce aphid populations on mustard only when aphids and parasitoids are coincident spatially and temporally (Weisser, 2000; Giles et al., 2003). However, in our case, it is not clear if the decline of aphid densities at the end was mainly due to the parasitoid activity, since aphid migration is high at that season. In a recent work, Athanassiou et al. (2003) reported that the reduction of *M. persicae* densities on tobacco in central Greece was not due to the activity of biocontrol agents, but mainly due to density independent factors. On the other hand, the coexistence of parasitoids (as in the case of our studies), acts chiefly as a stabilizing factor, that “protects” the system from collapsing (Lei and Hanski, 1997; Hanski, 1999). However, it is generally expected that competition reduces parasitization rate (Kavallieratos et al., 2002a). In case of parasitoid coexistence, different aphidiine species seem to utilize host groups with different characteristics (size, location etc.), leading to spatial segregation and the development of local, independent populations. While this fact has been examined extensively for parasitoids of lepidopterous larvae (Lei and Hanski, 1997, 1998; Hanski, 1999) it has not been attempted for aphid parasitoids.

Thus it can be concluded from the above that further studies on the coexistence of parasitoids should be focused on detailed search for the cause of their spatiotemporal trends. Practically, the study of the effect of parasitoids on the reduction of aphid numbers, their distribution on different plant parts and the factors that determine this effect, may help mustard growers to incorporate natural enemy thresholds into aphid management, which will reduce the insecticidal applications and promote sustainable crop protection.
Fig. 6.1 Average (± SE) number of *L. erysimi* and per cent mummified *L. erysimi* per sampling unit (average value) during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.2 Average (± SE) number of *L. erysimi* and per cent mummified *L. erysimi* per terminal 10 cm twig during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.3 Average (± SE) number of *L. erysimi* and per cent mummified *L. erysimi* per middle leaf during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.4 Average (± SE) number of *L. erysimi* and per cent mummified *L. erysimi* per lower leaf during *Rabi* 2010 (A) and 2011 (B).
**Fig. 6.5** Average (± SE) number of mummies and per cent hyperparasitized mummies by *P. aphidis* per sampling unit (average value) during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.6 Average (± SE) number of mummies and per cent hyperparasitized mummies by *P. aphidis* per terminal 10 cm twig during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.7 Average (± SE) number of mummies and per cent hyperparasitized mummies by *P. aphidis* per middle leaf during *Rabi* 2010 (A) and 2011 (B).
**Fig. 6.8** Average (± SE) number of mummies and per cent hyperparasitized mummies by *P. aphidis* per lower leaf during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.9 Average (± SE) number of *D. rapae* and *A. colemani* per terminal 10 cm twig during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.10 Average (± SE) number of *D. rapae* and *A. colemani* per middle leaf during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.11 Average (± SE) number of *D. rapae* and *A. colemani* per lower leaf during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.12 Relative composition of parasitoids, *D. rapae*, *A. colemani* and hyperparasitoids *P. aphidis* per sampling unit during *Rabi* 2010 (A) and 2011 (B).
Fig. 6.13 Relative composition of parasitoids, *D. rapae*, *A. colemani* and hyperparasitoids *P. aphidis* per terminal 10 cm twig during *Rabi* 2010 (A) and 2011(B).
Fig. 6.14 Relative composition of parasitoids, *D. rapae*, *A. colemani* and hyperparasitoids *P. aphidis* per middle leaf during Rabi 2010 (A) and 2011 (B).
Fig. 6.15 Relative composition of parasitoids, *D. rapae*, *A. colemani* and hyperparasitoids *P. aphidis* per lower leaf during *Rabi* 2010 (A) and 2011 (B).
Table. 6.1 Correlation of observed variables with different weather parameters during *Rabi* 2010 and 2011

<table>
<thead>
<tr>
<th></th>
<th>Max. Temp. (°C)</th>
<th>Min. Temp. (°C)</th>
<th>Temp. mean</th>
<th>Rain (mm)</th>
<th>Av. Wind Speed (kmph)</th>
<th>RH mean</th>
<th>BSS</th>
<th>Eva. (mm)</th>
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<tbody>
<tr>
<td><strong>2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total <em>L. erysimi</em></td>
<td>0.31</td>
<td>0.62</td>
<td>0.31</td>
<td>0.00</td>
<td>0.64</td>
<td>-0.42</td>
<td>0.41</td>
<td>0.09</td>
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<tr>
<td>Per cent <em>D. rapae</em></td>
<td>0.63</td>
<td>0.62</td>
<td>0.64</td>
<td>-0.20</td>
<td>0.24</td>
<td>-0.62</td>
<td>0.67</td>
<td>0.76</td>
</tr>
<tr>
<td>Per cent <em>A. colemani</em></td>
<td>0.42</td>
<td>0.44</td>
<td>0.44</td>
<td>-0.17</td>
<td>0.27</td>
<td>-0.54</td>
<td>0.57</td>
<td>0.53</td>
</tr>
<tr>
<td>Per cent total parasitism</td>
<td>0.65</td>
<td>0.63</td>
<td>0.65</td>
<td>-0.20</td>
<td>0.21</td>
<td>-0.61</td>
<td>0.66</td>
<td>0.78</td>
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<tr>
<td>Per cent total hyperparasitism</td>
<td>0.57</td>
<td>0.52</td>
<td>0.56</td>
<td>-0.15</td>
<td>0.05</td>
<td>-0.38</td>
<td>0.44</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>2011</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Total <em>L. erysimi</em></td>
<td>0.09</td>
<td>0.43</td>
<td>0.24</td>
<td>0.26</td>
<td>-0.24</td>
<td>0.63</td>
<td>-0.16</td>
<td>-0.39</td>
</tr>
<tr>
<td>Per cent <em>D. rapae</em></td>
<td>0.89</td>
<td>0.74</td>
<td>0.85</td>
<td>-0.23</td>
<td>-0.38</td>
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<td>Per cent <em>A. colemani</em></td>
<td>0.92</td>
<td>0.77</td>
<td>0.89</td>
<td>-0.23</td>
<td>-0.42</td>
<td>-0.48</td>
<td>0.62</td>
<td>0.78</td>
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<tr>
<td>Per cent total parasitism</td>
<td>0.87</td>
<td>0.73</td>
<td>0.84</td>
<td>-0.22</td>
<td>-0.37</td>
<td>-0.48</td>
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<tr>
<td>Per cent total hyperparasitism</td>
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<td>0.67</td>
<td>0.78</td>
<td>-0.24</td>
<td>-0.31</td>
<td>-0.50</td>
<td>0.55</td>
<td>0.75</td>
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</tbody>
</table>
DISCUSSION

The main purpose of the present investigations was to probe into the soundness of our taxonomic knowledge of *D. rapae* a parasitoid of *L. erysimi*. Also an attempt was made to study the seasonal occurrence, distribution and sampling indices for the parasitoid *Diaeretiella rapae vis-a-vis* its host, *Lipaphis erysimi*.

The results of the present study are discussed under the following headings:

7.1 Taxonomic placement of *D. rapae*

Modern day molecular studies also support this placement (Sanchis *et al.*, 2000). Currently family Braconidae comprises of two reasonably well defined group of subfamilies *viz.*, cyclostomes including the subfamilies Microgastrinae, Cheloninae, Helconinae etc., most of which are ectoparasitic and a relatively less well defined group of endoparasitic subfamilies *viz.*, Alysiinae, Braconinae, Rhyssalinae, Doryctinae etc. grouped together as non-cyclostomes. The relationship of Aphidiinae to other subfamilies of Braconidae and also the interrelationship between the various tribes under Aphidiinae make the position of Aphidiinae in this broad scheme unclear (Belshaw and Quicke, 1997) thus has been placed between a basal cyclostome grade and a derived non-cyclostome lineage (Quicke and Achterberg, 1990). However it still remains unclear to which of the many braconid subfamilies the Aphidiinae are most closely related (Belshaw and Quicke, 1997) but no doubt exists about the placement of subfamily Aphidiinae under family Braconidae (Wharton *et al.*, 1992).

A critical analysis of the existing literature shows that the classification of Aphidiinae which we see today is the culmination of efforts for over a century (Table 13). Subfamily Aphidiinae was erected by Forester in 1862 and placed under family Braconidae. Prior to Forester’s classification members of this subfamily were placed under Ichneumonidae (Linnaeus, 1758). Thereafter this subfamily was raised to the family level (Haliday, 1833; Mackauer and Stary, 1967; Mackauer, 1968; Takada, 1968; Stary, 1970; Stary and Ghosh, 1983). Westwood (1840) also agreed to their being raised in the hierarchy but included them under family Flexiliventres. However, Forester (1862), Cresson (1880), Ashmead (1900), Smith (1944), Wharton *et

Four main tribes, viz., Ephederini, Praini, Aphidiini and Trioxini have been recognized within Aphidiinae based on adult and larval morphology (Mackauer, 1968; O’Donnell, 1989; Finlayson, 1990). As with its position within the Braconidae, the tribal relationships within the Aphidiinae have proved intractable (Mackauer, 1961; Cho, 1984; Gardenfors, 1986). Two of the tribes, the Aphidiini and Trioxini, are united by the possession of a higher derived female reproductive system (LaRalec, 1993) and embryology (Tremblay and Calvert, 1971). Relationships with the other two tribes are problematic as these appear to be plesiomorphic in different characters: the Ephedrini in wing venation, and the Praini in venom gland apparatus (Edson and Vinson, 1979) and pupation habit. Although Stary (1960, 1966, 1970, 1976, 1988) contributed substantially to our knowledge of Aphidiinae but he did not attempt any further subdivision of this subfamily. Modern molecular phylogenetic analysis have also proved inconclusive, indicating the presence of either three viz., Ephederini, Praini and Aphidiini (Smith et al., 1999; Sanchis et al., 2000) or four viz., Ephederini, Praini, Aphidiini and Trioxini (Belshaw and Quicke, 1997) or five viz., Ephederini, Praini, Monoctonini, Trioxini and Aphidiini (Sanchis et al., 2000) tribes wherein all recognized tribes are monophyletic (Sanchis et al., 2000; Smith et al., 1999).

*D. rapae* was originally described by McIntosh in 1855 as *Aphidius rapae* thereafter this genus has been described as *A. vulgaris* by Bouche, 1834; *A. rapae* by M’Intosh, 1855; *A. (Trionyx) rapae* by Curtis, 1860; *Diaeretus chenopodii* by Foerster, 1867; *Trioxys piceus* by Cresson, 1880; *Lipolexis chenopodiaphidis* by Ashmead, 1889; *A. brassicae* by Marshall, 1896; *D. californicus* by Baker, 1909; *D. nipponensis* by Viereck, 1911; *D. obsoletus* by Kurdjumov, 1913; *D. napus* by Quilis, 1931; *D. croaticus* by Quilis, 1934; *D. plesiorapae* by Blanchard, 1940; *D. aphidum* by Mukherji and Chatterjee, 1950: 4-6. Finally Genus *Diaeretiella* was erected by Stary (1960) the species *rapae* was transferred to this genus. From the beginning all the above genera have been always placed under family Braconidae and subfamily Aphidiinae irrespective of the controversies regarding the placement of the subfamily Aphidiinae within the family Braconidae, or the tribal relationships within the Aphidiinae.
7.2 On the concept of the genus *D. rapae*

Genus *Diaeretiella* was erected by Stary (1960) with the following characters: head transverse, as wide as or wider than thorax at tegulae; antennae filiform, with variable number of segments; eye of medium size; mandibles bidentate. Notaulices developed on the fore part of mesoscutum. Propodeum distinctly areolated. Fore wing with pterostigma triangular; metacarp longer than width of pterostigma; radial vein developed, not longer than $\frac{2}{3}$ of its possible length; otherwise venation effaced beyond basal cell towards the apex except cubital cell 2 and indicated part of cubital vein. Hind wing with complete basal cell. Abdomen of female lanceolate; ovipositor sheaths and ovipositor straight or slightly curved upwards, sparsely haired and the species *rapae* was transferred to this genus.

However these characters were found to be much more variable during the current studies than has been previously supposed and thus the morphological basis upon which the nominal species has been distinguished is unsatisfactory. Although while redescribing *D. rapae*, Stary (1961) gave morphometric details of a few taxonomic characters like “… temple as wide as transverse eye-diameter; gena as wide as 1/3 to 1/7 of longitudinal eye-diameter; tenterio-ocular line 1/4 to 1/6 as long as intertentorial line; F1 and F2 of equal length, 2.5 times as long as wide; tergite I about 3 to 3.5 times longer than wide at spiracles…” but he did not quantify many important characters like: length of last flagellomere/length of flagellomere I; length of last flagellomere/width of last flagellomere; tenterio-ocular line/inter tentorial line; length of median longitudinal carina on propodeum/length of dorsal carina on first tergum; width of central areola/width of mesopleuron; length of fore wing/maximum width or fore wing; width of stigma/length of stigma; length of ovipositor sheath/length of tergite I; length of ovipositor sheath/width of ovipositor sheath at base and number of lateral carinae on tergite I.

Present studies show that the number of antennal segments varied by two in males and three in females. The number of lateral carina on anterolateral area of petiole varied widely between nine to fifteen. Central propodeal areola shows considerable variability in width and shape, sometimes is even subdivided into 2 to 3 subareola. The tenterial index ranges from 0.27-0.45 compared to previously published range of 0.17-0.25. Gena was observed to be as wide as 0.42-0.46 of
longitudinal eye-diameter as compared to the previously published ranges of 0.14-0.20. Previously published description recorded F1 and F2 of equal length and 2.5 times as long as wide but the present studies quantify F1 and F2 as 2.77±0.18 mm and 2.76±0.17 mm. Ratio of width of pterostigma to length of pterostigma ranges from 0.26-0.34, as compared to previously published range of 0.20-0.25. Tergite I was previously described as about 3-3.5 times longer than width at spiracles, but presently it was recorded to vary in the range of 2.36-2.73.

The description of *D. rapae* during the present studies has been further strengthened with the following additional characters, *viz.*, two new propodeal characters namely, median longitudinal carina length and central areola width; several new wing parameters like, metacarp (*R*1) length, radial length, M basal length, distance between tip of r and apex of wing, distance between tip of r and base or *R*1, distance between base of stigma and r, M+Cu length and maximum width of the first sub discal cell etc to quantify the variability in the propodeal region and the wing.

7.3 Morphological variability of different populations of *Diaeretiella rapae* (M’intosh)

Eleven populations of *Diaeretiella rapae* reared from parasitized *Lipaphis erysimi* were collected from nine different places of India among which six populations were collected on mustard from Almora, Delhi, Deradhun, Indore, Nainital and Pantnagar and five populations parasitizing *L. erysimi* were collected on cabbage from Aligarh, Almora, Barapani, Delhi and Pantnagar.

During the current studies forty quantitative characters of two kinds i.e. two meristic and thirty-eight ratio characters were found to be crucial in defining *D. rapae* were used. Further to encompass the variability in size and shape of wings, more emphasis was placed on the fore wing characters so ten ratio characters from the fore wing were selected.

Forty quantitative characters of two kinds i.e. two meristic and thirty-eight ratio characters were found to be crucial in defining *D. rapae* at all its taxonomic levels were used. To encompass the variability in size and shape of wings, more emphasis was placed on the fore wing characters with the selection of ten ratio characters from fore wing. Sixteen characters were found with CV values higher than 10 and hence can be considered as valuable character for discriminating different
populations, among these sixteen characters ratio of intero orbital distance to intero ocellar distance was found with lowest CV value of 10.32, hence is expected to have minor role in discriminating different populations. Ratio of length of pronotum to length of mesonotum was recorded with highest CV value of 28.88, hence is expected to contribute significantly in differentiating the populations. Analyses of measurements showed that, three ratio characters viz. length of flagellomere I to width of flagellomere I; width of mesopleuron to length of mesosoma and length of M+Cu to length of 1Cua had heavier principal component weights in the first principal component and served as the main contributing variables in the diagnostic differentiation of the populations.

Analyses of measurements showed that, three ratio characters *viz.* LF1/WF1 (Length of flagellomere I/width of flagellomere I), WMP/LMES (Width of mesopleuron/length of mesosoma) and LMCu/L1CuA (Length of M+Cu/length of 1Cua) had heavier principal component weights in the first principal component and served as the main contributing variables in the diagnostic differentiation of the populations. Following cluster analysis two groups were formed: Group 1 dominated by population from plains and Group 2 dominated by population from higher altitudes. *D. rapae* reared from *L. erysimi* infesting cabbage in Aligarh and Delhi were found as the most closely related populations in the first group, with population from Pantnagar mustard joining as a sister group to the two. In the second group population from Nainital mustard and Pantnagar cabbage were placed as the most closely related populations, with Almora mustard joining as a sister group to them. From our study it was hypothesized that being widely distributed in different parts of India, with wide host range, formation of host races as well as geographical races in *D. rapae* appears to be a remote possibility although some variability may be observed between the populations of plain and hill region.

7.4 Seasonal occurrence, distribution and sampling indices for *Lipaphis erysimi* and its parasitoids on mustard

Field studies to assess the seasonal occurrence and the spatial distribution of parasitoids of *Lipaphis erysimi* (Sulzer) on mustard, *viz.*, *Aphidius colemani* Viereck, *Diaeretiella rapae* (M’Intosh) conducted at the research farm of Indian Agricultural Research Institute, New Delhi, India (28°4'N, 77°09'E and 223.06m above mean sea level) during two consecutive *rabi* seasons of 2009-10 and 2010-11 indicated that *A.*
Colemani and D. rapae were the principal parasitoid species of L. erysimi on mustard at Delhi. The relative abundance of A. colemani and D. rapae differed between the two years. The seasonal abundance, hyperparasitic potential and distribution of P. aphidis (Bouche) (Pteromalidae: Hymenoptera), a hyperparasitoid of D. rapae, also differed during the two years.

The density of L. erysimi individuals were higher on the terminal 10 cm shoot of the plants compared to those collected from the leaves in the middle and lower part in both years but without significant difference. In contrast, the numbers of mummified L. erysimi individuals were higher on leaves collected from the middle part of the plants than on those from the terminal 10 cm shoot and lower part in both years.

During both the years, i.e., crop season of 2009-10 and 2010-11 the aphid count per plant increased rapidly since their first appearance in the field. A sharp decline in aphid count per plant was observed after the 10th standard week during 2010 and 12th standard week during 2011. This could have been due to the relatively high mean maximum temperature of the preceding week which was 31.3°C and 30.9°C in 2010 and 2011 respectively. Due to this sudden increase in temperature and other unfavourable conditions like crowding and poor host plant quality, alate adults of aphids were produced and migration followed leading to the sudden crash in aphid population (Hardie and Lees, 1985; Chapman, 1998). During both the years highest per cent mummification due to parasitism was observed on the last date of sampling when mean aphid densities per plant were very low. Hence, the results of the present study may suggest that parasitoids did not reduce aphid densities in either of the years tested, given that the aphid presence was high for most of the sampling period. This could be attributed to their delayed action since, in agreement with Stary (1988), the first mummies generally appeared after the increase of aphid population density to high levels. Our study also revealed the same trend as there was a three weeks lag period in 2010 and two weeks lag period in 2011 between the colonization by aphids of the first plant and occurrence of the first mummified individuals. However, the decreasing trend in aphid population which coincided with the increasing per cent mummification during the first half of March during both years suggests that parasitoids do regulate the aphid population partially, at least, at the latter half of the mustard growing season. The higher percentage of mummification observed on the leaves collected from middle part of the mustard plant during both years could be attributed to the production of honeydew on the more populated terminal parts of the
plant which restricts oviposition by the parasitoids through their immobilization (Stary, 1970). Furthermore, aphids in large colonies increase at such a high rate that the parasitoids can attack only a small percentage of them (Stary, 1970).

During our study higher number of mummified aphids was found on the leaves collected from the middle part of the plant as compared to the either terminal 10 cm shoot or leaves collected from lower half of the plant. During both the years *D. rapae* was always found to be more abundant on middle leaves as compared to terminal 10 cm shoot and lower leaves whereas, *A. colemani* preferred to remain on terminal 10 cm shoot in greater number than in other parts of the plant. Within plant distribution of *A. colemani* mummies on mustard is similar with the host behavior of another species of *Aphidius*, viz., *A. nigripes* Ashmead, a parasitoid of *Macrosiphum euphorbiae* (Thomas) on *Solanum tuberosum* L., where mummies were found particularly on the apical stratum of the plant. Brodeur and McNeil (1991, 1992) offered three possible explanations for the above in the apical parts of the potato plants: (a) parasitoids mummifying far from the aphid colony would be in sites with less honeydew and thus could avoid the detection by some predators, such as coccinellids as honeydew acts as a kairomone for several aphid predators (Ben Saad and Bishop, 1976); (b) the activity of the hyperparasitoids is restricted by the solar radiation. Further, hyperparasitoids being very tiny are subject to hydrothermic stresses when exposed to insolation for extended periods (Willmer and Unwin, 1981); (c) the solar radiation reduces the length of pupal development and thus shortens the period the mummies are exposed to natural enemies. Observations similar to the present results in mustard, the within plant distribution of *M. persicae* mummies on tobacco, have been reported by Lykouressis and Mentzos (1995). However, observations during the present studies indicated that mummies were found mostly within the aphid colony but some evidence was also there of movement by parasitized aphids which eventually fall on the upper side of the middle leaf. Surprisingly in most of such cases mummies were found to be parasitized by *A. colemani*. This is in confirmation with the views of Muller *et al.*, 1997 who had opined that (a) many but not all aphid species leave the colony after parasitization (b) hyperparasitoids number were significantly higher on the leaf collected from middle half of the mustard plant where they were not exposed directly to the solar radiation. Thus, parasitoids suffered high levels of hyperparasitization on the middle leaves of the plants given that
mummified aphids were more abundant on them compared to the terminal 10 cm shoot and lower leaves during both years of the study; (c) solar radiation seems to benefit A. colemani mummies but not D. rapae mummies since these “prefer” the shaded places of the tobacco plants. Generally, the plant architecture may influence interactions over several trophic levels (Brodeur and McNeil, 1991). Another possible explanation for the differential mummification on terminal 10 cm shoot middle and lower leaves is that parasitized aphids often drop from their feeding site and mummify on middle or lower leaves (Chow and Mackauer, 1999).

One additional complication in assessing the spatial synchronization between aphids and parasitoids is the lack of spatio-temporal synchrony. Apparently, parasitoids can reduce aphid populations on mustard only when aphids and parasitoids are coincident spatially and temporally (Weisser, 2000; Giles et al., 2003). However, in our case, it is not clear if the decline of aphid densities at the end was mainly due to the parasitoid activity, since aphid migration is high at that season. In a recent work, Athanassiou et al. (2003) reported that the reduction of M. persicae densities on tobacco in central Greece was not due to the activity of biocontrol agents, but mainly due to density independent factors. On the other hand, the coexistence of parasitoids (as in the case of our studies), acts chiefly as a stabilizing factor, that “protects” the system from collapsing (Lei and Hanski, 1997; Hanski, 1999). However, it is generally expected that competition reduces parasitization rate (Kavallieratos et al., 2002a). In case of parasitoid coexistence, different aphidiine species seem to utilize host groups with different characteristics (size, location etc.), leading to spatial segregation and the development of local, independent populations. While this fact has been examined extensively for parasitoids of lepidopterous larvae (Lei and Hanski, 1997, 1998; Hanski, 1999) it has not been attempted for aphid parasitoids.

Thus it can be concluded from the above that further studies on the coexistence of parasitoids should be focused on detailed search for the cause of their spatiotemporal trends. Practically, the study of the effect of parasitoids on the reduction of aphid numbers, their distribution on different plant parts and the factors that determine this effect, may help mustard growers to incorporate natural enemy thresholds into aphid management, which will reduce the insecticidal applications and promote sustainable crop protection.
Aphids as pests are known worldwide. Their biological peculiarities along with extensive monoculture and indiscriminate use of agricultural chemicals etc. have complicated matters further which have in turn stimulated research on their biological control. *Diaretiella rapae* (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) is the most common parasitoid associated with various species of aphids in all the major agro ecosystems and has been described as one of the most important factor for natural control of mustard aphid, *Lipaphis erysimi* (Kaltenbach). Although very minute in size this parasitoid has been recorded from many parts of our country. Considerable work on its hosts, seasonal occurrence and parasitism have been carried out but a holistic approach involving emphasis on both ecological and taxonomic studies has not been applied to this cosmopolitan species. Therefore in depth studies were undertaken on *D. rapae* not only to quantify the morphological variations present but also to study the inter and intra- plant distribution of *D. rapae* at field level.

The first chapter introduces the subject matter on the genus *D. rapae* indicating its distribution, economic importance and salient characters. The state of our knowledge with regard to this parasitoid, in brief, is also elucidated.

All the pertinent literature on the biosystematics of parasitoid *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) *vis-a-vis* its aphid host has been reviewed critically under various subheadings in the second chapter. In this, a chronological account of the work done by various workers is given. Emphasis has been laid more on work done in the Indian subcontinent.

The source of the material, which formed the basis of the investigations and the procedure, adopted for collection, mounting and preparation of the material for final microscopic studies along with details of the statistical analysis comprises the third chapter.

The fourth to sixth chapters are in the form of research papers entitled, “Redescription of *Diaeretiella rapae* M’Intosh (Hymenoptera: Braconidae:
Aphidiinae) with emphasis on the variability of diagnostic characters”; “Comparison of eleven populations of Diaeretiella rapae (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) from India” and “Seasonal occurrence, distribution and sampling indices for Lipaphis erysimi (Hemiptera: Aphidoidea) and its parasitoids (Hymenoptera: Braconidae: Aphidiinae) on mustard”

The salient results of the three research papers are as follows:

- *D. rapae* has been redescribed with several additional characters supported by adequate illustrations.

- Altogether 300 specimens from eleven populations collected from different parts of India were examined.

- Forty quantitative characters of two kinds i.e. two meristic and thirty-eight ratio characters were found to be crucial in defining *D. rapae* at all its taxonomic levels were used.

- To encompass the variability in size and shape of wings, more emphasis was placed on the fore wing characters with the selection of ten ratio characters from fore wing.

- Sixteen characters were found with CV values higher than 10 and hence can be considered as valuable character for discriminating different populations, among these sixteen characters ratio of inter orbital distance to intero ocellar distance was found with lowest CV value of 10.32, hence is expected to have minor role in discriminating different populations.

- Ratio of length of pronotum to length of mesonotum was recorded with highest CV value of 28.88, hence is expected to contribute significantly in differentiating the populations.

- Analyses of measurements showed that, three ratio characters viz. length of flagellomere I to width of flagellomere I; width of mesopleuron to length of mesosoma and length of M+Cu to length of 1Cua had heavier principal component weights in the first principal component and served as the main contributing variables in the diagnostic differentiation of the populations.

- Following cluster analysis two groups were formed: Group I dominated by population from plain and Group II dominated by population from higher
altitudes. *D. rapae* populations reared from *L. erysimi* collected on cabbage from Aligarh and Delhi were placed as the most closely related populations in the first group, with Pantnagar mustard population joining as a sister group to the two. In the second cluster *D. rapae* populations reared from *L. erysimi* collected on mustard from Nainital and from Pantnagar on cabbage were placed as the most closely related populations, with Almora mustard joining as a sister group to them.

- Thus it was hypothesized that being widely distributed in different parts of India, with wide host range, formation of host race as well as geographical race in *D. rapae* appears to be a remote possibility although some variability may be observed between the populations of plain and higher altitude.

- High densities of *L. erysimi* were found in the second fortnight of February during both the crop seasons, viz., 2009-2010 and 2010 - 2011. The density of *L. erysimi* individuals were higher on the terminal 10 cm twig of the plants compared to those collected from the leaves in the middle and lower part of the plant during both years but without significant differences.

- During both the years two parasitoids, *viz.*, *A. colemani* and *D. rapae* on *L. erysimi* and a hyperparasitoid, *Pachyneuron aphidis* on the parasitoids were observed.

- The mummification rate due to the two parasitoids showed an increasing trend late in the season (first fortnight of March), and the per cent mummification reached 53.18% at the end of the season during 2010 whereas in 2011 it reached 65.5%. Although, the number of mummified *L. erysimi* individuals were higher on leaves collected from the middle part of the plants than on those from the top 10 cm twig and lower part during both years but it was statistically insignificant.

- The relative abundance, parasitic/ hyperparasitic potential and distribution of *A. colemani* and *D. rapae* and *Pachyneuron aphidis* varied during the two years.

- This is followed by a brief discussion on the systematic position of the genus *D. rapae* along with its redescription, its distribution vis-à-vis its host *L. erysimi*, other parasitoids/ hyperparasitoids present in the field.
• The results of the present studies on the distribution of both the pest and the parasitoids/ hyperparasitoid on the plant will help identify a suitable sampling unit and a natural enemy threshold which may lead to a rational aphid management in mustard.
Biosystematic studies on the parasitoid *Diaeretiella rapae* (M’Intosh) (Hymenoptera: Braconidae) *vis-a-vis* its aphid host

**Abstract**

Aphids as pests are known worldwide. Their biological peculiarities along with extensive monoculture and indiscriminate use of agricultural chemicals etc. have complicated matters further which have in turn stimulated research on their biological control. *Diaretiella rapae* (M’Intosh) (Hymenoptera: Braconidae: Aphidiinae) is the most common parasitoid associated with various species of aphids in all the major agro ecosystems and has been described as one of the most important factor for natural control of mustard aphid, *Lipaphis erysimi* (Kaltenbach). Although very minute in size this parasitoid has been recorded from many parts of our country. Considerable work on its hosts, seasonal occurrence and parasitism have been carried out but a holistic approach involving emphasis on both ecological and taxonomic studies has not been applied to this cosmopolitan species. Therefore in depth studies were undertaken on *D. rapae* not only to quantify the morphological variations present but also to study the inter and intra-plant distribution of *D. rapae* at field level.

Forty quantitative characters including two meristic and thirty-eight ratio characters from 167 specimens belong to 11 populations collected from nine different parts of India along with 8 figures of the various diagnostic characters were used to redescribe *D. rapae*.

To validate the morphometric characters utilized in the present study, statistical analyses were undertaken for evaluating the extent of variation within or among the populations. Based on their CV values, sixteen characters were found to be essential for discriminating different populations among which ratio of length of pronotum to length of mesonotum was recorded with very high CV values and thus expected to contribute significantly in differentiating the populations. Analyses of measurements showed that, three ratio characters *viz.* length of flagellomere I to width of flagellomere I; width of mesopleuron to length of mesosoma and length of M+Cu to length of 1CuA had heavier principal component weights in the first principal component and served as the main contributing variables in the diagnostic differentiation of the populations.

Following cluster analysis two groups were formed: Group I dominated by populations from plains and Group II dominated by populations from higher altitude. Populations of *D. rapae* reared from *L. erysimi* collected on cabbage in Aligarh and Delhi appeared as the most closely related populations in the first group, with the population from Pantnagar mustard joining as a sister group to the two while in the second group population reared from *L. erysimi* on mustard from Nainital and cabbage from Pantnagar were placed as the most closely related populations, with the population from Almora mustard joining as a sister group to them. From our study it is hypothesized that being widely distributed in different parts of India, with wide host range, formation of a host race as well as a geographical race in *D. rapae* appears to be a remote possibility although some variability may be observed between the populations of plains and higher altitude.

*D. rapae* has been recorded extensively by numerous researchers but its distribution *vis-a-vis* its host *L. erysimi*, other parasitoids/hyperparasitoids present in the field has not been assessed. Since information about the distribution of both the pest and the parasitoid on the plant helps identify a suitable sampling unit and a natural enemy threshold which may lead to a rational aphid management in mustard field studies were conducted, during two consecutive *rabi* seasons *viz.*, 2009-10 and 2010-11, in order to assess the seasonal occurrence and the spatial distribution of *L. erysimi* *vis-a-vis* its parasitoids/hyperparasitoids.
Samples were collected at weekly intervals since the first colonization of mustard plants by aphids (2nd standard week and 3rd standard week in 2010 and 2011 respectively) and continued until the last harvest of plants (11th standard week and 12th standard week in 2010 and 2011 respectively). During both the years’ two parasitoids, viz., *D. rapae* and *Aphidius colemani* Viereck, along with a hyperparasitoid *Pachyneuron aphidis* (Bouche) (Pteromalidae: Hymenoptera) were recorded on *L. erysimi* on mustard (*Brassica juncea* variety Pusa bold) at IARI. Generally, high *L. erysimi* densities were found during the second fortnight of February for both years. The mummification rate showed a specific increasing trend late in the season i.e. first half of March. In 2010, the percentage of mummification reached 53.18 per cent at the end of the season whereas in 2011 it was as high as 65.5 per cent. Although the density of *L. erysimi* individuals were higher on the terminal 10 cm twig of the plants compared to those collected from the leaves in the middle and lower parts in both years but statistically no significant difference was there. In contrast, the numbers of mummified *L. erysimi* individuals were higher on leaves collected from the middle part of the plants than those from the top 10 cm twig and lower part during both the years. However the relative abundance of *A. colemani* and *D. rapae* differed during the two years. Seasonal abundance, hyperparasitic potential and distribution of *P. aphidis*, was also discussed.

The high densities of beneficials that are often reported to occur in mustard fields clearly suggest that an integration of natural enemies into aphid management is feasible, and these agents may play a key role in an Integrated Pest Management based strategy.
पर्जीविक साहित्य विभेदिता राजी (एम' इंटोश) (हायमेनोटेगा या बैकोनआयडी) का इसके एफिय अविभेद के साथ में जैववर्गीकरण संबंधी अध्ययन

सार

विवेक भर में एफिय की पीड़ा के बाद जाना जाता है। उनके जीवनभर सभी विभिन्नताओं के साथ-साथ सम्बंध नामकरण एवं कृषि और भूमिकाय रास्तों आदि के अध्याय यथा प्रवाह ने विचार को और अधिक जटिल बना दिया है जिसमें उनके जीविक नियंत्रण पर अनुसंधान की महती आवश्यकता है। सभी कृषि-पारिस्थितिक तंत्रों में एफिय की पूर्णतियों के साथ संबंध डायमेनोटेगा या बैकोनाेयडी एफियडेनी) सबसे आम पर्जीविक है तथा इसे रसायन के एफिय, लायरेस्स इरीमामॉ काल्प) के प्राकृतिक नियंत्रण हेतु सर्वाधिक महत्वपूर्ण कारकों में से एक, वर्णित किया गया है। यद्यपि यह पर्जीविक, परमाणु में अत्यंत मुख्य है, इसे हारे आतिवेद, उत्तर संबंधी प्रकार एवं पर्जीविक के विचार में काफी कार्य हो चुका है कितना इस सर्वाधिक पूर्णतियों पर कितना संकेतित उपयोग जिसमें पारिस्थितिक एवं गर्भीकरण संबंधी अध्ययनों दोनों पर जोर दिया गया हो का अनुप्रयोग नहीं किया गया है। इसलिए ठाणे राजी पर एक गांव अध्ययन किया गया जो न केवल इसके विश्वास आकारिक विभिन्नताओं के प्रामाणिक हेतु किया गया वर्तमान सार पर ठाणे राजी के अंतर एवं अंतर-पारिपत्रिक वितरण के अध्ययन में भी किया गया।

ठाणे राजी के पुनर्वर्तनार्थ भारत के नौ भिन्न भिन्न क्षेत्रों से लगभग 300 नूतन/11 आबादियों को दो मेरिटिक एवं अड़तीम अनुपात गुणों सहित चारों मात्रानुक्रम गुणों के साथ-साथ अन्य निदानमूर्त गुणों संबंधी 8 चित्रों का उपयोग किया गया है। आबादियों के मध्य एवं उनके भीतर भिन्नता की सीमा के मूल्यांकन हेतु साँख्यिकीय विश्लेषण ने इस अध्ययन में प्रयुक्त आकारिकता गुणों के साथ में उपयोग किया गया। उनके से ती मानों के आधार पर, विभिन्न आबादियों में स्थित करने के लिए मोटे गुण आवश्यक पाए गए जिनमें एफिय नेवला की लम्बाई का मूल्य प्राप्त किया गया और इस प्रकार से आबादियों कि बीच में भिन्न करने में शायद इसका महत्वपूर्ण योगदान होता है। मानों के विश्लेषणों ने दर्शाया कि तीन अनुपात गुणों में फलोनॉप्सीयर की लम्बाई का फलोनॉप्सीयर की वैल्ड के साथ मूल्य मूल्यगृहों की मोटाई का मूल्यॉमा की प्रमाणण के साथ तथा एम + सी यू की लम्बाई का 1 सी यू ए की लम्बाई के साथ अनुपात प्रथम प्रथम घटक में भारी प्रमुख घटक था। तथा आबादियों में निदानमूर्त विभेदन हेतु इन परिवर्तनों में प्रमुख योगदान दिया।

समर्थ विश्लेषण के पश्चात दो समूह ने समूह 1 समलग्न और गुणों में प्राप्त आबादियों की प्रभाविता वाला तथा समूह 11 अधिक संबंधों में प्राप्त आबादियों की प्रभाविता वाला। पहले समूह में, अलगए तथा दिल्ली में ठाणे राजी के इसामियरा पर पली एवं पत्ताखों पर एकाधिक आबादियों सबसे निकट संबंधी आबादियों प्रतिनिधित हुई, पश्चात रसायन में प्राप्त आबादि सहित नैनिकल से मानों एक, इसीमामियरा पर पली और रसायन पर एकाधिक आबादि तथा पत्ताखों पर पत्ताखों पर एकाधिक आबादियों सबसे निकट संबंधी आबादियों के रूप में साथ रखी गई। हमारे अध्ययन से यह अवधारणा बनती है कि भारत के विभिन्न भागों में विस्तृत वितरण
सरसों के वर्गों में अक्सर मिलने वाले लाभकारियों के उच्च बनने वाले स्थान के रूप में दर्शाते हैं कि एफिड प्रवेश के इसके पारितिक श्रेणियों का समक्ष श्रेयस्कर है तथा ये कारक समक्ष पीड़क प्रवेश आधारित रणनीति में मुख्य भूमिका निभा सकते हैं।


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