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Measuring Genetic Variation in Natural Enemies Used for Biological Control: Why and How?

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Introduction

Biological control programmes of pests with the use of natural enemies have been employed for more than a century. However, on average, only between 10 and 35% of the introduced natural enemies established successfully (Force, 1967; Huffaker and Messenger, 1976; Hall and Ehler, 1979), and only a fraction of these led to economic control (Mackauer, 1972; Hall *et al.*, 1980). There is thus considerable room for improvement and, as pointed out by Roush (1990a) and Hopper *et al.* (1993), the importance of genetic aspects as a means of improving the success of this crop protection strategy has always attracted a great deal of attention (Mally, 1916; Simmonds, 1963; Mackauer, 1976; Messenger *et al.*, 1976; Hoy, 1985, 1992; Roush, 1990b). The central issue in this context is based on the analysis of genetic variation in the key biological attributes of the biocontrol agents (Roush, 1990a). An important effort of research has been devoted repeatedly to the quantification of the *inter*specific variability of some of these attributes, and results are supposed to provide some help in selecting the right species for controlling an identified pest in a given environment (Messenger and van den Bosch, 1971). Other works have aimed at describing the *intra* specific genetic variability in the biological traits studied. In this case, most authors tried to compare different populations of the same species originating from different geographical locations (Simmonds, 1963; Messenger and van den Bosch, 1971; Diehl and Bush, 1984; Caltagirone, 1985). The aim of comparing different populations of the same species is usually to identify the strain that is most adapted to the environment where it will be released, and thus to improve the success of the biological control programme (Messenger and van den Bosch, 1971). Finally, some work has been done to quantify the *intra*specific, *intra*population genetic variabil-

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ity in the biological attributes of the natural enemies, but only little effort has been made at this level of genetic variation (Lewis *et al.*, 1990; Hopper *et al.*, 1993).

The present chapter will discuss the intrapopulation level of genetic variation in natural enemies, and more specifically insect parasitoids. Genetic comparison between populations will not be considered here, nor will studies that assessed genetic variation by analysing laboratory populations generated by the amalgamation of geographically different stains (e.g. Simmonds, 1947; Parker and Orzack, 1985; Antolin, 1992a,b). Not all biological characteristics are polygenic (i.e. influenced by many loci, each of small effect), but such an assumption is probably true for most traits important for the efficiency of natural enemies in biological control programmes (Roush, 1990a). Thus, only intrapopulation genetic variability in continuously variable, polygenic traits will be considered here. Genetic variation in molecular markers (enzymatic or nucleic) will thus not be discussed, nor variability in pesticide resistance that is repeatedly considered to be due to allelic variation at just a few major genes (Roush and McKenzie, 1987; Hoy, 1990a,b).

Among all potential natural enemies that can be used for pest control, insect parasitoids are the most important. They can be found in nearly all ecological systems, and there are probably almost 2 million species on Earth (Godfray, 1994). Most of them reproduce through arrhenotoky, a special kind of parthenogenesis in which mated females can produce either unfertilized eggs, giving rise to haploid males, or fertilized eggs, leading to diploid females. This peculiar haplo-diploid reproductive system often precludes application of the usual procedures for the estimation of quantitative genetic variation (Carton *et al.*, 1989; Sequeira and Mackauer, 1992). However, although a low level of genetic variability is expected within parasitoid populations (Crozier, 1977; Hoy, 1990a; Legner, 1993), some authors have argued that, as in any insect species, some significant genetic variation should be found (Ayala, 1982; Bartlett, 1985), especially when behavioural traits are being quantified (Barinaga, 1994; Pompanon *et al.*, 1999).

After discussing why it is usually considered to be of prime importance to quantify the intrapopulation genetic variability in the biological attributes of insect parasitoids, the different experimental and statistical methods that can be used to quantify such a level of genetic variation in quantitative biological traits will be presented. Then, an exhaustive analysis of the results available in the literature will be discussed. In a final part, the choice of the biological attributes that need to be studied for better pest-control efficacy by the parasitoids will be considered.

Why Measure Intrapopulation Genetic Variation in Natural Enemies?

Despite the fact that genetic variability in mass-produced and released natural enemy populations remains poorly described, an accurate estimation of such genetic variation has always been considered to be important, for several complementary reasons. As pointed out by Remington (1968), a population introduced in a new environment will have to cope with a community composed largely of organisms not present in its origin location. It will therefore interact with environmental constraints for which individuals' genotypes will not have been previously selected. Thus, in order to maximize its ability to establish, it is generally admitted that the released population of natural enemies should have the maximum possible genetic variability (Simmonds, 1963; Force, 1967; Mackauer, 1972; Lewis *et al.*, 1990; Wajnberg, 1991). In order to check this, such genetic variation has to be quantified accurately.

Measuring the level of genetic variation in biological attributes of natural enemies should also lead to a better estimate of their ability to evolve in response to the environmental characteristics of the system used to rear and produce them. Since colonies maintained and produced in the laboratory usually experience constant environments that are obviously different from those encountered in the field, selection could occur for laboratory-adapted genotypes. The response to selection will be more rapid when genetic variation is high. In turn, such selection might rapidly reduce both the genetic variance in the biological traits involved (Bulmer, 1976) and the pest-control efficacy during field release (Mackauer, 1972, 1976; Bartlett, 1984; Joslyn, 1984; Roush, 1990b; Hopper *et al.*, 1993).

During the production phase of insects in the laboratory, some individuals can contribute randomly to the next generation more than others, and this sampling effect can lead to a random loss of some alleles in each generation. This process is called genetic drift, and can lead to a substantial reduction in the genetic variability within the reared population (Joslyn, 1984; Roush, 1990b; Hopper *et al.*, 1993). However, it should be noted that genetic drift is important only in small populations, and theoretical developments indicate that a loss in genetic variability due to such a process is not always a matter of concern (Nei *et al.*, 1975; Hopper *et al.*, 1993). As pointed out by Wajnberg (1991), even if this loss is sometimes of minor importance, it cannot be prevented. At best, it can be reduced as much as possible. For this, an accurate quantification of the genetic variability in the reared population can also provide some fruitful information. More generally, measuring the genetic variation in important attributes of the natural enemies should allow us to better define the rearing conditions used to produce them before field release (Boller, 1979).

As a general rule, an accurate quantification of genetic variability should lead to better estimates of the survival potential of the founder population during the rearing and production process (Mackauer, 1972), and in the field after being released (Hopper *et al.*, 1993). An accurate estimation of the intrapopulation genetic variation should thus help in better defining how new natural enemies' biotypes should be found and collected, and also to optimize methods and timing used to release them.

Perhaps the most discussed reason why it is important to measure the genetic variability in biological traits within populations of natural enemies is that the existence of significant genetic variation can allow commencement of a breeding selection programme aimed at improving the efficacy of the released animals to control the target pests (Hoy, 1976; Wajnberg, 1991). Such an improving selection procedure is equivalent to that used for domestic animals and plants (Wilkes, 1947). In the case of natural enemies, the traits that are the subject of improvement should be related to insectary production and/or to field effectiveness (Hoy, 1976). The usefulness of the method has been discussed by several authors (for a review, see Mackauer, 1972, 1976), but some arguments against selection breeding of natural enemies have also been raised on several occasions. As a general rule, a breeding selection programme of a strain of natural enemies is usually considered a difficult task (Simmonds, 1963), and White *et al.* (1970) thus recommended the use of selection for improving pest-control efficacy only when there is no chance of finding a better-adapted species. Moreover, the laboratory procedure employed to select an improved strain will probably reduce genetic variation, which goes against the need to release a population showing the maximum possible genetic variability (Wilson, 1965). Also, the idea of successfully selecting a natural enemy for improving its pest-control efficacy supposes an accurate understanding of the different biological attributes that determine effectiveness, a task which is repeatedly considered as extremely difficult (Wilson, 1965; Hoy, 1976; Mackauer, 1976). Finally, successfully selecting a population for an accurately identified attribute could lead to the possible occurrence of correlated, pleiotropic variations in other biological traits (Simmonds, 1963). As a result, the character selected might indeed be improved, but some undetected accumulation of other characteristics, which might be disadvantageous in the field, could appear.

In the past few decades an important body of data has accumulated on the ecological characteristics of insect parasitoids. For this, optimal foraging theory was used, allowing us to identify the optimal biological characteristics these insects should adopt in order to maximize their reproductive potential (Stephens and Krebs, 1986; Godfray, 1994). Such a theoretical approach provided information of prime importance for defining what the optimal features of a potential biocontrol agent should be (Waage, 1983, 1990). However, talking about optimal biological traits supposes that these traits have been settled progressively by natural selection, and thus that there is/was, in the natural enemy population, genetic variability upon which natural selection could act. Quantifying such intrapopulation genetic variability would thus also provide important information for confirming the relevance of the theory and its ability to produce results useful for improving the efficacy of biological control programmes.

Methods for Measuring Intrapopulation Genetic Variation

Variation among individuals, for any kind of quantitative trait, is a common feature of all biological studies (Bartlett, 1985; Lewis *et al.*, 1990). Moreover, in most cases, measuring such variation is considered to require experimental protocols based on many replicates to collect solid estimates of means (Roitberg, 1990). Such phenotypic variability is known to be the result of interactions between the genotype, or genetic make-up, of each organism and the environment that it lives in (Collins, 1984). The genetic source of variability is passed from individuals to their progeny, which is not the case for the environmental (i.e. non-genetic) source of variation. The aim of the methods to be presented briefly here is both to separate these two sources of variability, genetic and environment, and to see whether the genetic part is of statistically significant importance in contributing to the phenotypic variation observed (Falconer, 1989).

The variability among all individuals in a population can be quantified with the so-called variance, and the notation V_P is used to describe the variance of the phenotypes. Using statistical models, such phenotypic variance can be divided into V_E, the variance due to environmental effects, and V_G, the variance of the genotypes. In turn, V_G can be further subdivided into the variance due to additive genetic effects (V_A), the variance due to dominance effects (V_D), and the variance due to the interaction between the loci involved in determining the trait under consideration (= epistasis) (V_I) (see, for example, Falconer, 1989, for a detailed presentation). So, the basic model used in this case is:

$$V_P = V_E + V_A + V_D + V_I \tag{2.1}$$

Of the three types of genetic variance, V_A is considered to be the most important, since it defines the breeding value for the trait on the organisms. In other words, selection – artificial or natural – will essentially act on V_A, and the methods that have been defined to estimate the genetic variability in quantitative biological traits are all built to estimate it, in a direct or indirect way.

In theory at least, perhaps the simplest way to estimate V_A for a given trait would be to measure two populations in several different environments. The first population would consist of mixed individuals of different genotypes, the other would consist of individuals all having the same genotype. Results obtained for the first population will enable us to estimate the total phenotypic variance, V_P. Those obtained for the other would give an estimate of the environmental variance, V_E, only. The difference between these two variances would give an estimate of the additive genetic component of the variance, V_A (Collins, 1984). However, in most cases, using such a simple method is not realistic and other more feasible, accurate methods are available. They will be presented briefly below. These methods are usually used to estimate the genetic variation in ecological (e.g. behavioural) traits in insect populations. However, they can also be used to quantify any kind of quantitative genetic variability, at the molecular, cytological, physiological or morphological levels (Parsons, 1980). They can be used to estimate the genetic variation, and thus to assess the genetic structure of natural and mass-reared populations, even those of species where details of the genome are not well known (Parsons, 1980).

Parent–offspring regression

If the variation in a quantitative trait is, at least in part, genetically determined, then offspring should resemble their parents. Based on this basic statement, Sir F. Galton, more than a century ago, proposed a study of the transmissibility of a trait over two successive generations. For this, the trait is quantified in a set of parents (mothers, fathers or both), and also in their progenies. Then, the slope of the regression line of the offspring's values on that of their parents is computed and its statistical significance tested. If both parents are quantified, their average value is used. Only one offspring can be measured for each parent, but, if several offspring are quantified, then their average value is also used to estimate the slope of the regression line. Finally, in this latter case, if the number of offspring is not constant, the regression can be weighted by the actual number of offspring measured for each parent (Falconer, 1989).

In most cases, only parasitoid females are useful for controlling a target pest in a biological control programme, and, thus, the trait studied can sometimes be measured in females only (e.g. fecundity, host attack rate, etc.). In this case, the parent–offspring regression analysis becomes a mother–daughter regression analysis.

Sib analysis

Another method consists of mating a number of males (sires) with a number of females (dams). Each sire has to be mated to more than one dam, but each dam is mated with one sire only. The trait under study is then measured in the offspring produced by the mated females. Finally, an analysis of variance (ANOVA) is used to quantify the variation among sires, among dams (within sires), and within the progeny of each dam. In turn, the estimated parameters of the ANOVA can be used to test the significance of the genetic variation among dams and among sires for the quantified traits.

Despite the fact that one advantage of this method is in excluding possible maternal effects through the comparison of different sires (Falconer, 1989; Hopper *et al.*, 1993), it has almost never been used to quantify the genetic variability in biological attributes of natural enemies.

Family analysis

A related, and more commonly used, method is the isofemale strains method, also called isofemale lines or family analysis (Parsons, 1980; Hoffman and Parsons, 1988). In this method, an array of families (or lines, or strains) is founded, each family from a single mated female, and the trait under study is quantified in several offspring produced by each female in the F_1 generation. Finally, a one-way ANOVA is used to test for a significant difference among average values of the different families compared, which will indicate a significant genetic variability in the quantified trait. The main difference between this method and the previous one is that females used to found each family are not mated with clearly identified males. On the contrary, they are supposed to be mated randomly.

A compromise should be found between the number of families compared and the number of individuals measured in each family. Of course, small or moderate-size datasets could lead to imprecision in estimating the genetic variation (Shaw, 1987; Falconer, 1989) and/or to poor power of the statistical test used to detect it. The number of replicates used usually depends on the time needed to measure each individual and how difficult it is to found and rear the different families compared.

Despite the fact that it could lead to laborious experimental protocols, the family analysis is considered to be more simple to use than the other methods available. However, the variation between families can be caused by a mixture of additive, dominance and epistatic components (Falconer, 1989). Hence, despite giving a crude estimate of the genetic variation in the trait studied, this method does not provide direct information regarding the ability of the population to respond to natural or artificial selection, and other more accurate methods should be used.

Finally, it has to be noted that the different families compared are just a sample of all possible families in the entire population studied. Therefore, the 'family' effect in the ANOVA used to test the difference among the families' averages values should be considered a random effect and should be treated accordingly. Occasionally, this might raise some problems, especially when generalized linear models are used for handling traits that are not distributed according to a Gaussian distribution (McCullagh and Nelder, 1991).

Breeding selection

Despite the fact that the modification of the average value of a trait through several generations of breeding selection does not accurately represent a way to quantify its genetic variability in a population, it proves that the observed phenotypic variation is at least partly under genetic control (Roush, 1990a). Such a procedure has thus been used by several authors to demonstrate the existence of significant genetic variation in several biological attributes of natural enemies. Briefly, two categories of methods can be used here. In mass selection, the individuals used to found the next generation are chosen according to their own phenotype. In family selection, the individuals are chosen according to the average value of the family from which they come (Collins, 1984; Falconer, 1989; Wajnberg, 1991).

All the methods presented above can be used to estimate the heritability of the trait studied, defined in either its narrow or its broad sense. In its narrow sense, this is the ratio of additive genetic variance to the total phenotypic variance. In the broad sense, it is defined as the ratio of the total genetic variance (i.e. additive, dominance and epistatic) to the total phenotypic variance (Hoffmann and Parsons, 1988; Falconer, 1989). Also, these different methods are sometimes combined together into a single experimental set-up. For example, a mother–daughter regression analysis can be performed over two successive generations, with several offspring measured for each mother. At the F_1 generation, the daughters represent different isofemale lines, which are compared by means of a family analysis (e.g. Chassain and Boulétreau, 1991; Bruins *et al.*, 1994). Of course, since the main aim of these methods is to estimate the genetic and environmental components of the phenotypic variation observed, they should all be conducted under conditions where environmental causes of variation are reduced to a minimum. Measurements have to be made in precisely controlled environmental chambers, under constant temperature, humidity, measured at the same age, and so forth. If possible, the method used to quantify the traits should be as simple, fast and cheap as possible in order to perform a large number of replicates in a short time interval. These points, or some of them, might be difficult to solve, and sometimes even be limiting factors.

Finally, some attributes of natural enemies cannot be quantified by a single value. For example, several quantitative parameters are sometimes needed to quantify a single behaviour of a parasitoid female. In this case, all the methods described above can be generalized using multivariate statistical methods that are built to take into account possible correlation among the different traits measured. For example, a multidimensional regression analysis (i.e. a canonical regression analysis) can be used to perform a multivariate parent–offspring regression (e.g. Wajnberg, 1993), or a factorial discriminant analysis can be used to compare the mean-vector describing all isofemale lines in a family analysis.

Intrapopulation Genetic Variation in Insect Parasitoids

A detailed survey was performed over the main scientific publication databases to find all references describing intrapopulation genetic variation in quantitative attributes of insect parasitoids. Only 39 references were found, covering 23 different species names (see Table 2.1). These 39 references were published over a period of almost 60 years (from Wilkes (1942) to Gu and Dorn (2000)), which represents a very low publication rate (about 0.67 publications per year). However, two-thirds of the references appeared during the past decade (1990–2000), suggesting increasing interest in this sort of scientific work.

The process of host exploitation by insect parasitoids is usually described using a series of steps that draw them progressively closer to their hosts and enable the immature stages to develop successfully in them (Vinson, 1975, 1976). A parasitoid female first has to discover a habitat where potential hosts are living. Then, she should discover a host, and should recognize and attack it. The laid progeny should be able to overcome or evade the host's

Table 2.1. List of all species in which intrapopulation genetic variation in quantitative traits was studied.

Family, species	References
Braconidae	
Aphidius ervi	Sequeira and Mackauer (1992), Henter (1995), Gilchrist (1996)
Asobara tabida	Mollema (1991)
Cotesia glomerata	Gu and Dorn (2000)
Cotesia melanoscela	Weseloh (1986), Chenot and Raffa (1998)
Microplitis croceipes	Prévost and Lewis (1990)
Ichneumonidae	
Aenoplex carpocapsae	Simmonds (1947)
Horogenes molestae	Allen (1954)
Microplectron fuscipennis	Wilkes (1942, 1947)
Eucoilidae	
Leptopilina boulardi	Carton *et al.* (1989), Perez-Maluf *et al.* (1998)
Pteromalidae	
Muscidifurax raptor	Geden *et al.* (1992)
Nasonia vitripennis	Orzack (1990), Orzack and Parker (1990), Orzack *et al.* (1991), Orzack and Gladstone (1994)
Trichogrammatidae	
Trichogramma brassicae	Chassain and Boulétreau (1991), Fleury *et al.* (1993), Wajnberg (1993, 1994), Bruins *et al.* (1994), Pompanon *et al.* (1994, 1999), Wajnberg and Colazza (1998)
as *Trichogramma maidis*	Chassain and Boulétreau (1987), Wajnberg (1989), Wajnberg *et al.* (1989)
Trichogramma cacoeciae	Chassain and Boulétreau (1991), Pompanon *et al.* (1994)
Trichogramma carverae	Bennett and Hoffmann (1998)
Trichogramma dendrolimi	Limburg and Pak (1991), Schmidt (1991)
Trichogramma evanescens	Limburg and Pak (1991), Schmidt (1991)
Trichogramma minutum	Urquijo (1950), Liu and Smith (2000)
Trichogramma pretiosum	Ashley *et al.* (1974)
Trichogramma semifumatum	Ashley *et al.* (1974)
Trichogramma voegelei	Mimouni (1991)
Scelionidae	
Telenomus busseolae	Wajnberg *et al.* (1999)
Tachinidae	
Lixophaga diatraeae	Pintureau *et al.* (1995)

internal defence mechanisms and, finally, the parasitoid must find the host nutritionally suitable for complete development, resulting in adult emergence. Figure 2.1 gives the distribution of the different biological traits in which an intrapopulation genetic variability has been studied along such a sequential process of host exploitation by parasitoids. There is a clear lack of studies on biological traits involved in host habitat location by the foraging parasitoid female, and on those involved in evading the host immune system by the laid progeny. This is probably due to the difficulties in measuring these traits in the laboratory and in performing all the replicates needed to identify significant genetic variation.

About 60% of these studies used the family analysis method (see Fig. 2.1). In this case, on average, 19.85 ± 3.70 families were compared, with an average of 12.15 ± 3.73 individuals measured in each family. The parent–offspring

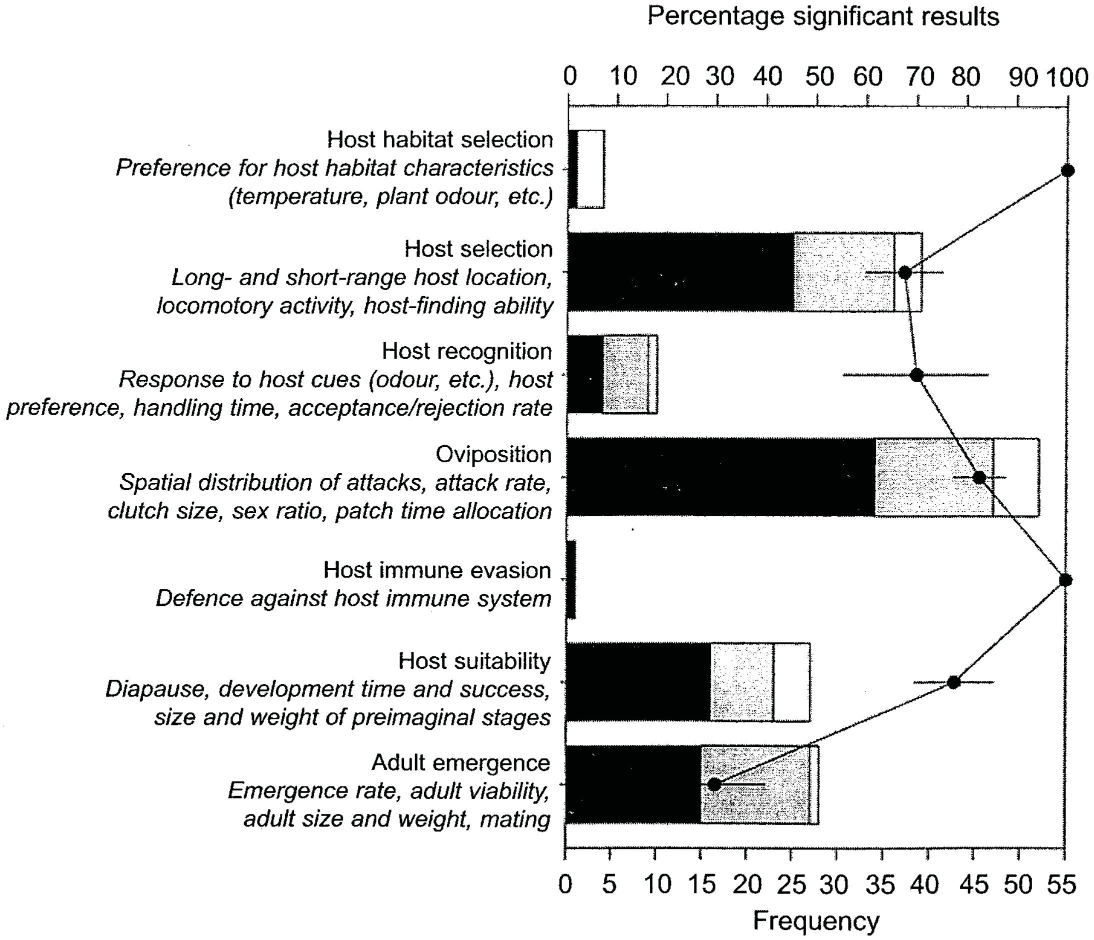

Fig. 2.1. Frequency distribution of the biological traits in which intrapopulation genetic variation has been studied in insect parasitoids. The seven classes of traits correspond to a series of steps usually used to describe the host exploitation process by parasitoids (from top to bottom). Black, grey and white rectangles correspond to the use of family analyses, parent–offspring regressions or breeding selection methods, respectively. The curve represents percentages (± SE) of these studies showing significant genetic variability.

regression method was used in 29.81% of the studies, with an average of 65.31 ± 13.22 parent/offspring couples measured. The remaining studies (i.e. 10.56%) used the breeding selection method. In this case, on average, 9.67 ± 1.50 generations of selection were followed.

About 70% of these studies showed the existence of significant genetic variation. However, as can be seen in Fig. 2.1, the percentage of significant results varies along the different steps of host exploitation by parasitoids, and this variation appears to be statistically significant (Fisher's exact test, $P < 0.005$). Some biological traits seem to present higher genetic variation than others. Traits that are closely related to fitness are often supposed to have been purged of genetic variance by strong directional selection, and hence should present lower genetic variability (Gustafsson, 1986; Mousseau and Roff, 1987; Roff and Mousseau, 1987; for an alternative point of view, see Price and Schluter, 1991; Houle, 1992). This should be the case for the life-history traits of parasitoids, such as body size and development time, and female fecundity. For such life-history traits, 61.76% of the studies showed the existence of significant genetic variation, while this percentage rose to 77.78% for the other biological attributes studied. The difference between these two percentages is statistically significant (one-sided Fisher's exact test, $P = 0.025$). Thus, the hypothesis that traits related to fitness should present lower genetic variation seems to be verified in insect parasitoids. Most of these traits belong to the 'adult emergence' (last) category shown in Fig. 2.1, and this might explain the corresponding decrease in the percentage of significant results obtained in this case.

What Characters Should be Studied?

As we have seen, methods that can be used to quantify the genetic variation in biological attributes of natural enemies are available and have been in use for several years now. However, identifying the biological features in which intrapopulation genetic variation should be studied still remains an open question. Ideally, the traits should be related to pest-control efficacy, and are thus important either during the laboratory production phase or after field release. This is related to the question of the quality of biocontrol agents (Bigler, 1989). As such, it has always been, and still is, an intensively debated issue, and lists of potential characters are found repeatedly in the literature (e.g. Flanders, 1947; Hoy, 1976, 1990b; Mackauer, 1976; Roush, 1979; Waage and Hassell, 1982; Hopper *et al.*, 1993). Briefly, based either on empirical intuition or on theoretical considerations, the main proposed categories of criteria are climatic adaptation, habitat preference, synchrony with hosts, host-searching capacity, specificity, dispersal ability, attack rate, female fecundity and sex ratio.

As a general rule, it has been admitted repeatedly that it is difficult to identify those biological attributes that distinguish efficient biological control agents from unsuccessful ones (DeBach, 1958; Hoy, 1976, 1990b; Roush, 1990a). Another difficulty is the interpretation of laboratory results and their link to field performance (Bigler, 1989). Some authors even assert that the important traits just cannot be identified satisfactorily (for a discussion, see Roush, 1979). Of course, the important attributes of a potential biological control agent should clearly depend on the nature of the pest to be controlled, on the ecological features of the crop to be protected, and also on the type of release (inoculative, inundative, etc.) that is to be used (Hoy, 1976; Roush, 1979). Thus, general statements would probably lead to misinterpretations (Bigler, 1989). Despite this, some authors argue that, in most cases, the important traits should be those implicated in the overall ability and propensity of the released insects to colonize new habitats (Force, 1967).

Identifying the important traits is usually considered to be based on a prerelease evaluation of natural enemies, a procedure that is often perceived more as an art than a science (van Lenteren, 1980). A possible, time-consuming process would be to find different strains or variants for every potential trait (or to create them through breeding selection experiments) and to study the relationship between their average phenotypic value, estimated in the laboratory, and their efficacy in controlling the targeted pest after being released in the field. This process has been used successfully by Bigler *et al.* (1988), who found a significant positive relationship between the walking velocity of the females of different *Trichogramma maidis* (= *T. brassicae*) strains, estimated in the laboratory, and their parasitism rate against the eggs of the European corn borer, *Ostrinia nubilalis*, in the field. This indicates that the walking speed of the parasitoid females should be an interesting trait, and its genetic variation should be quantified in this wasp species (Pompanon *et al.*, 1994).

Finding the most important trait probably needs a costly, labour-intensive process. The use of mathematical models might provide substantial help in determining these traits before the experimental work (Bigler, 1989). To our knowledge, Wajnberg and Colazza (1998) provided the only example relating a modelling approach demonstrating the importance of a biological trait (i.e. the prospected surface per time unit of foraging parasitoid females) to an analysis of its intrapopulation genetic variation. More generally, theoretical approaches using optimality models are now used intensively in order to quantify the importance of foraging decisions in the reproductive ability of insect parasitoids. The traits that appear to be important are probably related to the pest-control efficacy of the parasitoids when they are used in biological control programmes (Waage, 1983, 1990). Such a theoretical approach allows us to identify the traits in which genetic variability should be studied. Among others, the traits that are revealed to be important are host attack rate, spatial aggregation of the attacks, selection of host patches, time allocation in host patches, etc. Wajnberg *et al.* (1999) provide the first attempt to demonstrate significant genetic variation in a trait (patch time allocation) that has been shown by theoretical approaches to be of importance in the reproductive ability of insect parasitoids.

Finally, there is no doubt that identifying the main biological attributes of natural enemies remains the most important issue for improving the efficacy of biological control programmes. It is also the most difficult question to solve and will certainly necessitate, in the future, the solution of numerous challenges, probably through the use of theoretical developments.

Conclusion

Only a limited number of studies of intrapopulation genetic variation in insect parasitoids have been published so far. So, as pointed out by Hopper *et al.* (1993), much work still remains to be done. A lot of information regarding the level of genetic variation in natural or mass-reared populations of natural enemies is still missing if we want to understand the ecology and evolutionary potential of beneficial insects used in biological control programmes. More accurately, the exhaustive bibliographic survey presented in this chapter indicates that some biological traits are studied less than others. For example, we have seen that the behavioural traits involved in the ability of an emerging female parasitoid to find a habitat where potential hosts are located still remain poorly studied. Actually, the research that is needed concerns the 'ecological genetics' of natural enemies, in the sense that the genetic variation should be studied in biological attributes that can be implicated in the ecology of their interactions with their hosts.

During the last decade of the 20th century, the development of new technologies to study numerous variable loci simultaneously led to the possibility of analysing, at the genome level, the genetic determinism of phenotypic variation. This so-called 'population genomics' approach (Black *et al.*, 2001) includes the creation of genetic maps enabling us to identify the major genes involved in the variation observed (i.e. quantitative trait loci, QTL). There is no doubt that such technology will allow us to quantify more rapidly and more accurately the genetic variation in populations of natural enemies. In turn, the results obtained will also provide the means to perform marker-assisted selection of their pest-control efficacy. These results will also certainly lead us to have a better insight into their evolutionary potentialities. The aim of this chapter is to stimulate research in this direction.

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