Chapter 12

RECENT PROGRESS AND POSSIBLE FUTURE TRENDS IN THE STUDY OF COCCINELLIDAE

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Ecology and Behaviour of the Ladybird Beetles (Coccinellidae), First Edition. Edited by I. Hodek, H.F. van Emden, A. Honěk.

A major objective of this book and this chapter is to stimulate new research; for this aim it is important not only to emphasize basic recent achievements, but also to question existing paradigms – and highlight the still unanswered problems.

Although in the Americas the coccinellids from the subfamily Epilachninae are important herbivorous pests and there represent the dominating economic focus, the worldwide emphasis is on Coccinellidae as biological control agents of sucking insects. The applied accent should not detract from the scientific interest and value of research on them that is not targeted directly to biological control. In any case, the economically-orientated material in this book needs setting in the wider context of coccinellids being only one component of a much larger complex of natural enemies discussed in Chapter 7. This makes their contribution to pest control very hard to isolate from that of the other taxa active in the relevant guilds (such as e.g. the aphidophagous guild).

In relation to the current increasing interest in conservation biological control, there are many attributes of coccinellids discussed in this book which should be assessed against the ecological and behavioural criteria needed for the different approaches which have been reviewed elsewhere (e.g. Barbosa 1998). With the relationships of ladybirds to habitats (Chapter 4) and food (Chapter 5), introducing single elements of diversity to improve biological control is probably more useful than increasing biodiversity *per se*.

In an even wider context, biological control is just one component of Integrated Pest Management (IPM). This began in the late 1950s as "integrated control" with increasing the ratio of natural enemies (including coccinellids) to aphids in alfalfa through reduced doses of insecticides. Biological control by coccinellids is likely to show positive synergism with both host plant resistance to the prey and insecticide, both of which are able to improve the numerical ratio of natural enemies to their prey (reviewed for IPM of aphids by van Emden 2007; see also Chapter 11.6.3 and Chapter 11.6.4).

The first two chapters, respectively on Phylogeny and Genetics, may appear out of place in relation to the title of this book. However, important recent progress has been made also in these fields, progress of which those interested in the biology and ecology of Coccinellidae should be aware as impacting on the interpretation of their results.

While the subfamilies of coccinellids are more or less worldwide in distribution, many tribes are restricted to particular biogeographical regions, and this has resulted in a number of alternative classifications (Vandenberg and Perez-Gelabert 2007). A comprehensive classification applicable to all geographical regions is needed. Some derived groups of species are often classified under a separate name, leaving the rest of the related species as a paraphyletic assemblage. Vandenberg and Perez-Gelabert (2007) propose that these sets of genera should be re-united or that paraphyletic genera be split to obtain a balanced classification. This can be only achieved by taxonomic revisions containing most described species. Phylogenetic relationships of genera, tribes and subfamilies and subsequent changes in higher classification must be obtained from analysis combining both morphological and molecular data. Mapping on cladograms of specific morphological and life history parameters, such as food specialization, possession of larval waxy exudations and defensive glands will allow us to understand which traits are homologies shared by descent and which are homoplasies achieved by parallel adaptations. Knowledge of the relationships of subfamilies and tribes within a family will allow better discrimination between general patterns and specific cases, as recommended by Sloggett (2005). This will enable us accurately to categorize groups between which comparisons are often made rather too facilely; for example, contrasts are often incorrectly proposed between aphidophagous (in fact almost exclusively Coccinellini) and coccidophagous coccinellids (in fact a polyphyletic assemblage from Chilocorinae, Scymninae and Coccidulinae). Contrasts between aphidophagous and coccidophagous ladybirds or other groups that differ in a specific trait should be analysed only between closely related species or genera (Chapter 1).

Methodological developments in the field of **molecular genetics and genomics**, including microarray technology and high-throughput sequencing, make molecular genetic studies of coccinellids much more achievable than they would have been even a few years ago. This applies equally to studies examining **gene expression** and to those using **molecular markers**. The wealth of data already available on coccinellid ecology and behaviour makes the group particularly well suited for research uniting both ecological and molecular approaches (Chapter 2). Aspects of coccinellid ecology that have already been the subject of intensive study from an evolutionary viewpoint, such as colour pattern polymorphism and male-killing, should particularly benefit from this approach. Our detailed knowledge of the **coccinellid genome** seems likely to increase by leaps and bounds in the next few years: it surely cannot be very long before the first whole coccinellid genome sequence becomes available.

What will be important is that the **research in molecular biology** does not end with the linking of genes with the insects' phenotypical characteristics, but **includes** the **biochemistry** by which the genes express their effect. Chapter 2 gives an account of some major areas where expansion of such research can make important new contributions. However, the presence of the DNA of **endosymbionts** (see below) can confuse the results and interpretation, and Chapter 2 explains the limits of using mitochondrial DNA from the body fluids of coccinellids. However, such genetic variation is still inherited and so subject to natural selection; this may well need to be included to explain the **genetic basis of behaviours** such as dietary specialization. It is when molecular techniques are targeted to identifying prey in gut contents or especially to determining phylogenetic relationships that techniques need to be sought that identify genetic markers on the nuclear chromatin of the particular species of prey or predator.

It seems very likely that **endosymbionts** are far more widely distributed in insects than we know at present, and it is time for a major assay of coccinellids to be carried out, both as regards their own symbionts and those of their prey. It could well be shown that some of the extraordinary **phenotypic plasticity** in the biology and behaviour (such as **food preferences** and **diapause behaviour**) may **not** have a **genetic basis** but reflect variation in the presence or titre of endosymbionts within genotypes. There are likely to be a great number of exciting new avenues of research in this emerging field of study; we should expect quite a lot of surprises.

One of the basic problems of the study of ladybird communities (Chapter 4) is that of **sampling**. Larvae are harder to spot than adults and are more nocturnal in activity, and so the many studies which count coccinellids visually at the same time as their prey collect a large number of zeros and singletons in distributions which are hard to define statistically and lead to dubious interpretation. Such dubious outcomes relate especially to the many studies seeking to compare populations in arable crops and more natural habitats. Two suggestions for future can be made. Firstly, **mark and recapture techniques** might be ideally suited to coccinellids. They could give much more accurate population estimates and comparisons of population density in different habitats. Moreover, good estimates of vagility can be obtained by repeated marking and recapturing. The point is indeed made in Chapter 5.4.1.5 that the long-distance searching behaviour of ladybirds needs further study and it is clear that there is growing interest in studying coccinellid movements from "natural" to arable crop habitats. This trend in research has developed from the increasing priority being given to a consideration of more natural habitats as "ecosystem services". Good estimates of ladybird **immigration and emigration** therefore become essential and it is surprising that mark and recapture studies have still been rather neglected.

A second comment about sampling relates to an almost universal use of sampling which matches **catch per unit effort** (i.e. numbers in so many sweeps, on so many plants etc.). A far better approach for coccinellids, since a large proportion of samples often return a zero value, is probably to measure the **reciprocal**, the effort needed for a unit catch (i.e. number of plants examined to reach a target number of coccinellids). Density per unit of measurement can still be calculated, but with similar accuracy of the results across the whole range of densities occurring during a season. The ultimate improvement in this direction is to design a sequential sampling plan based on some preliminary sampling. This enables the decision to be made on each sampling occasion as to whether or not more samples need to be taken to determine population size to a given level of accuracy. Sequential sampling plans (Southwood 1966) do require quite a lot of preliminary work, but should be seriously considered for long-term studies in one agroecosystem.

There are two limitations of current work on coccinellid communities. Because of the difficulties in publishing **raw community data** in "high standard" journals, the majority end up in regional or local publications. It is a pity that this information is not collected and subjected to meta-analysis! The second drawback is that the quality of species determination is usually not very high in other groups than the tribe Coccinellini.

With food interrelations, probably most research progress has been made in the field of **behaviour during foraging** (Chapter 5.4). Fifteen years ago most authors considered the foraging movements as completely random, but there is now much laboratory evidence that ladybirds are guided both by **olfactory and visual cues** (Chapter 5.4.1.2). Also, the first studies on the relevant **receptors** have been published. Quite a breakthrough has been the discovery of **oviposition deterrence**, as this gives us insight into decision-making by female coccinellids, something that was previously quite a mystery. However, field assays on the action of volatiles, both from plants and prey, are still largely missing, together with the research on the mutual interference among volatiles (synergy v. inhibition).

It is stressed in chapters 5.2.2 and 5.2.11 that **food specificity** should be considered from the view of physiological/nutritional suitability: the food is either **essential**, enabling reproduction and development of larvae, or **alternative** that just prevents starvation. Although this principle difference has been accepted by leading coccinellid researchers, in some papers the authors limit themselves to the vague terms suitable v. non-suitable food and the distinction is wrongly based on acceptability. It has been shown here that ladybirds may feed also on toxic food.

It is now timely to revisit some of the results on coccinellids which were obtained many years ago. An unfortunate consequence of scientists relying almost exclusively on computer searches of the literature is that they are quite likely not to encounter highly relevant papers published in the 1950s and 1960s and even earlier. Thus studies demonstrating great differences between results at constant and **alternating conditions**, as for example found in the relationship between temperature and coccinellid **voracity** (Chapter 5.3.1), do not seem to be remembered today. The chapters in this book report many studies carried out in growth rooms controlled at constant temperature and humidity, but very few indeed where the experiments were conducted at fluctuating conditions, particularly reflecting night/day variation. As pointed out above, the effect of such fluctuating conditions on coccinellid voracity is quite large; what other attributes of coccinellids are similarly affected?

Although usually a very long phase of the life cycle, **diapause/dormancy**, has lately attracted much less research activity than other topics. This is unfortunate, because the timing of dormancy is essential for the modern ecological approaches to biological control (conservation, augmentation) and for IPM: we must know when the ladybirds need to be helped by providing attractants and alternative foods at their spring arrival on crops (Chapter 6.3.1.5), which includes the period when the impact of coccinellids is often decided. Such information – also factors affecting survival during dormancy – are missing for most potentially effective coccinellid species. Apart from these applied aspects, diapause of coccinellids is a useful model for research in several fields (Chapter 6.5), e.g. the regulation of voltinism, i.e. annual number of generations (Chapter 6.2.16), or the ethological aspects of the flight to hibernation sites (Chapter 6.3.1.3).

Natural enemies of ladybirds (such as e.g. parasitoids) exert stronger and more persistent effects on the populations of phytophagous than on those of entomophagous Coccinellidae; also coccidophagous coccinellids may be more affected than aphidophagous ones. Phytophagous species, being typically **sedentary,** are more easily located by their enemies (especially by parasitoids) than are the **more mobile** predatory species of Coccinellidae. This explanation may also hold true for the greater susceptibility to natural enemies of coccidophages, since these are less mobile due to the relatively stable occurrence of their prey. Regardless of the coccinellid group considered, there is not much evidence for **top-down regulation** of their populations by natural enemies. Chapter 8 shows clearly that further studies are needed to clarify the role that natural enemies of ladybirds play in population dynamics of these predators. For ladybirds, as for their sternorrhynchan prey, parasitization has again proved easier to measure than predation.

Coccinellids depend on **semiochemical-mediated information** for important steps in their life history (Chapter 9). However, so far there is only a limited understanding of the mechanisms involved and of how chemical ecology interacts with other stimuli regulating life processes. Several of the chemical cues influencing mobility and foraging are commonly-occurring substances (such as green leaf volatiles and semiochemicals from other insects), and one of the future challenges is to understand the subtle mechanisms that contribute to the specificity of their activity for the individual coccinellid species.

This book presents the evidence, probably assembled for coccinellids for the first time, that the potential for **manipulating coccinellid behaviour with semiochemicals** has recently shown itself to have a practical future. However, as it seems unlikely that these chemicals can provide a grower-acceptable level of control on their own, it is important that development of techniques for using semiochemicals will be accompanied by the development of strategies for how they could be integrated with other approaches into an IPM package. Chapters 5.4.1 and 9 deal with active **behaviour-controlling molecules** as singletons, but the history of sex pheromone research has taught us that such compounds are often dramatically **synergized by triggering molecules,** which have little or no effect on their own. There is an example of this in alfalfa, with the attraction of chrysopid adults to a particular breakdown product of tryptophan in aphid honeydew; however, there is no response to the compound involved (indole acetaldehyde) unless the insects first perceive a volatile synomone from alfalfa (Hagen, 1986).

Although repeated evidence has accumulated on the effects of ladybirds in the natural control of several homopteran taxa, it may seem surprising that precise numerical data of their impact are hard to find, particularly on aphids in arable crops (Obrycki et al 2009). These problems are analysed in detail in Chapter 10 and 11, but three important ones are 1) that, as already mentioned at the start of this chapter, coccinellids are only one of over a dozen insect families which may be simultaneously predating on sucking insects in crops, 2) the technical difficulties of quantifying predation (as opposed to parasitism) and 3) the interaction in the field between predators and their prey is affected by several physical factors which vary with year.

Laboratory feeding studies (Chapter 10.2) remain a useful approach for determining prey acceptability and suitability for coccinellids, and consumption assays produce estimates of potential impact (Chapter 10.3). However, the strength of laboratory studies usually lies in providing a **base for interpreting field observations**. Traditional methods of assessing the impact of coccinellids on focal prey species (Chapter 10.4) continue to be invaluable and the best insights are usually obtained through a combination of approaches selected for their suitability for **particular prey-plant systems**, as each has inherent limitations. Although molecular analyses have advanced our understanding of the trophic relations of predatory arthropods, including the Coccinellidae, they have yet to yield data that accurately **quantify predation** in the field. The better studies have linked population surveys with molecular gut-content analysis to strengthen inferences about trophic relationships and mechanisms of prey specificity, as opposed to simply generating binary data on the presence/absence of target prey in predator guts. Semi-quantitative estimates of predation have been made using gut-content analysis by estimating the concentration of recognizable prey proteins in gut samples by ELISA (Chapter 10.7) or of prey DNA by quantitative PCR. Such studies provide valuable insights into the relative amount of recognizable material in the predator at a given time, but **do not offer 'quantitative' estimates of predation** because the number (and/or biomass) of prey consumed and the time elapsed since consumption cannot be inferred. Thus, although **molecular gut-content analyses** offer particular benefits for elucidating trophic interactions, they remain fraught with interpretive sources of error. Nevertheless, when used in combination with quantitative data from field experiments, they can generate unique insights that cannot be obtained with other approaches.

In the end, however, the data from assays of predator voracity have to be included in a population dynamics exercise in the field. At present, even were we able to measure voracity independently for all the groups of aphid predators in the crop, we do not have a satisfactory numerical approach to analysing the population dynamics of the aphids themselves in the field. The emphasis for IPM needs to move from manipulating coccinellid density to manipulating coccinellid : aphid ratios, which are much more amenable to quantification than "predatory impact".

The role of introduced coccinellids in **classical** biological control programmes (Chapter 11.3.1 & 11.3.2) has **diminished** as unanticipated impacts of exotic species have come to light (Chapter 11.3.3 & 11.3.4). Consequently, regulatory authorities are likely to permit only highly specialized species for use in classical programmes. Certain species will remain important for **augmentation** in specialized contexts (Chapter 11.4), but few novel augmentation applications for coccinellids have as yet been developed. The future appears brighter for **improving conservation** and **enhancing the efficacy** of naturally-occurring species in the field (Chapter 11.5). Advances will hinge on **improved holistic understanding of the ecological roles** of coccinellids and their ability to complement other beneficial species. This information is critical for the development of novel approaches to **habitat management** (Chapter 11.5.3) that could improve the efficiency of established coccinellid guilds in particular agroecosystems and enhance their ability to track economically important prey species in time and space.

It is clear from the chapters in this book and Chapter 11.6.3 that **coccinellid/host plant resistance**

interactions are still a relatively blank canvas with great research opportunities, though as mentioned earlier there is already quite a lot of evidence that the **positive synergism** between ineffective biological control and ineffective host plant resistance can lead to dramatic reduction of aphid populations.

Finally, a concern raised in several of the chapters is that **differences in experimental protocols** of different researchers make it difficult to develop an overall picture when integrating results from the literature. We do not seek to select which approach should be uniformly adopted for each experimental situation. More realistically, a major contribution that this book can make is to identify where inconsistencies exist and provide the literature review material to allow workers in future to evaluate how best to proceed to make their work of maximum utility to their colleagues. This might even extend to taking data additional to those required for the aim of the experiment.

One thing we need to stress in conclusion is that, whatever merit there may or may not be in the suggestions we have made in this final chapter, the most exciting future research breakthroughs concerning coccinellids will, by definition, be matters we have not thought of!

ACKNOWLEDGEMENTS

We gratefully acknowledge that several authors of individual chapters have contributed ideas to this overview. The discussion in this chapter follows the order of the other chapters in the book.

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