Monitoring the spread of an invasive alien

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Introduction

As a result of human activity, alien species of organism are reaching areas far outside their native ranges with ever increasing frequency. Many of these alien species, whether introduced intentionally or accidentally, fail to establish for a variety of reasons, but some do and these can have profound effects on native species and ecosystems. Much of the research on invasive species has focused on plants. While many invasive animal species are known, the precise manner in which they establish and spread has rarely been closely scrutinised, largely because problems consequent upon their establishment were only recognised some time after their arrival. This was the case with the invasive coccinellid, *Harmonia axyridis*, in North America. This ladybird, which was intentionally introduced into the USA many times in the twentieth century, as a biological control agent of aphids, became established in the 1980s. Now, many in North America regard it as a pest. More recently, *H. axyridis* has been introduced and become established in continental Europe. Again the early establishment of the species was not well monitored. However, in 2004, the species reached Britain. That year, a nation-wide alert was put out for sightings of *H. axyridis*, and funding was gained to monitor the species from the year of its establishment. In this paper we describe the methods used to monitor the spread of *H. axyridis* in Britain, give a first report of progress, and outline areas of research to be pursued.

The history of *H. axyridis*

Harmonia axyridis is endemic to Asia, being found from central Russia eastwards to Korea, Mongolia, China, Taiwan and Japan, and south into the Himalayas (Hukusima & Kamei, 1970; Sasaji, 1971; Kuznetsov, 1997). It feeds on a wide array of foods, but is primarily aphidophagous. Due to its relatively large size (approximately 7cm long), its voracious appetite for aphids and its wide habitat colonisation ability, it has been introduced for biological control purposes into many parts of the world, and in some has become established. In North America, the first attempt at release of *H. axyridis* was as early as 1916, but the species failed to establish (Gordon, 1985). Subsequently, repeated releases have been made in various parts of North America, but it was not until the 1980s that it finally became established. In 1988, breeding populations were recorded in Louisiana (Chapin & Brou, 1991). Whether these colonies resulted from stocks released for biocontrol, or were the result of accidental immigration has not been ascertained (Tedders & Schaefer, 1994; Day *et al*., 1994). Since 1988, the species has spread rapidly across most of the United States and into Canada, increasing in number, so that it has become the predominant aphidophagous coccinellid over much of North America (Tedders & Schaefer, 1994; Dreistadt *et al.*, 1995; Smith *et al.*, 1996; Colunga-Garcia & Gage, 1998; Hesler *et al.*, 2001; Koch, 2003).

Purely as a biocontrol agent, *H. axyridis* has been a success in North America. It has reduced damage inflicted by aphids and coccids to various crops, including pecan nuts, apple and citrus orchards, sweet corn, alfalfa, cotton, tobacco, winter wheat, soybean, strawberry and red pine. However, a variety of negative effects have also been disclosed. These include adverse effects on non-target prey (Koch *et al*., 2003), and on beneficial aphidophagous and coccidophagous insects, through both intra-guild competition and intra-guild predation (Brown & Miller, 1998; Colunga-Garcia & Gage, 1998; Michaud, 2002).

Harmonia axyridis has also become a nuisance to many people because of its tendency to overwinter in very large numbers on or in buildings in the autumn. On warm days, particularly towards the end of winter, the ladybirds become active (Huelsman *et al*., 2002). If disturbed, they reflex bleed, secreting an acrid-smelling yellow fluid, which stains soft furnishings. They also sometimes bite people, particularly in the autumn when they are trying to feed up for the winter. There have been instances of people developing an allergic rhinoconjunctivitus to *H. axyridis* (Huelsman *et al*., 2002; Magnan *et al*., 2002; Yarborough *et al*., 1999). Finally, *H. axyridis* sometimes damages crops. In the later summer, when building up reserves for the winter, it is attracted to ripe fruit, and will suck juice. This can cause blemishes to the fruit, reducing its economic value. In some Canadian and U.S. wineries large numbers of *H. axyridis* are attracted to ripe grapes and are harvested and crushed with the crop. Then, their acrid defensive alkaloids and pyrazines can seriously taint the bouquet and flavour of the wine produced (Ratcliffe, 2002; Ejbich, 2003).

Since 1982, *H. axyridis* has been available commercially in Europe for use as a classical biocontrol agent (Katsoyannos *et al*., 1997; Iperti & Bertand, 2001). It has been intentionally released in a number of countries in continental Europe (Iperti and Bertrand, 2001; Adriaens *et al*., 2003) and North Africa (S. El Nagdy, pers. comm.). Since about 2000, it has become established in Belgium, northern France, Holland, Germany, Luxemburg and Switzerland (Iperti & Bertand, 2001; Adriaens *et al*., 2003; P. Brakefield, pers. comm.; M. Majerus, pers. obs.; M. Kenis, pers. comm.).

Given the proximity of the French, Belgium and Dutch coasts to England, it was almost inevitable that *H. axyridis* would reach Britain.

The arrival of *H. axyridis* in Britain

The first identified individual of *H. axyridis* was a single melanic male found in Essex on 19th September 2004. This ladybird was identified by Majerus, who, in the light of reports of adverse effects of this ladybird in North America, prepared a press release detailing its arrival. The press release was circulated on 5th October. It began:

"The Ladybird Has Landed

A new ladybird has arrived in Britain. But not just any ladybird: this is *Harmonia axyridis*, the most invasive ladybird on Earth.

Harmonia axyridis, which is variously called the Harlequin ladybird or the Multi-coloured ladybug, is a deadly threat to a suite of insects, including butterflies, lacewings and many other ladybirds."

The tone of this release had the desired effect, and caught the imagination of both the media and the general public. Four national newspapers, both the BBC and Independent television and three national radio stations, in addition to numerous local newspapers, magazines and radio stations covered the story. Through the media, Majerus and his research group, in collaboration with Dr Helen Roy of Anglia Polytechnic University, asked the public to look out for *H. axyridis* and to send in any 'odd-looking' ladybirds that they found. The response from the public was overwhelming. Sightings and specimens of *H. axyridis* started to come in within a week. It soon became apparent that the Essex male was not alone. The species was shown to be widely distributed in the south-east of Britain, with breeding colonies reported from several localities (London, Essex, and the Kent and Norfolk coasts) (Majerus *et al*., in press a).

In addition to the response from the public, scientists from the Biological Records Centre, and Rothamstead Research Centre, with an interest in coccinellids, suggested collaborative research. Short-term funding to employ a project officer who would collate incoming records, and develop online recording was gained from DEFRA through the National Biodiversity Network and NERC. Collaborations with the Biological Records Centre, RSPB, and the Natural History Museum in London were established.

On 15th March 2005, the Harlequin Ladybird Survey was formally launched at the Natural History Museum, London. Again media interest was considerable, and the survey was featured on the front page of The Times newspaper.

The main aims of the survey were to monitor the spread of *H. axyridis* in Britain and to assess its impact on native coccinellids. In consequence, two websites allowing online recording were established. The Harlequin Survey website, http://www.harlequin-survey.org, contains information about the arrival of this species in Britain, how to recognise it, its ecology and how to submit records, either online or by post. The second website, at http://www.ladybird-survey.org, is designed to allow recording of all other British ladybirds, so that the impact of the spread of *H. axyridis* on its competitors can be assessed.

Records sent in by the public are of two types: verified or unverified. A verified record is one supported by either a specimen sent to one of the project staff, or a photograph of sufficient quality to allow unequivocal identification. Only verified records are used in distribution maps or other analyses. Unverified records are kept on the database, and members of the project team follow up those of particular interest.

The website draws particular focus to ten native species of British ladybird, which are being monitored regularly in a number of locations. Records of these species collected in 2005 will provide benchmark data against which the densities of the species in the same locations can be compared over the next five years. For this element of the survey, people living in regions that *H. axyridis* has yet to reach, in addition to those in areas where it is already known, have been targeted. The ten species chosen have been selected on the basis of their principal food and habitat specialisation, so that the impact of *H. axyridis* on ladybirds with a variety of characteristics can be appraised (Table 1).

Table 1: Species of ladybird selected to ascertain the impact of *H. axyridis*, on native ladybirds in Britain. Species were selected to encompass a range of habitat and dietary preferences. (Adapted from Majerus & Roy, 2005.)

Species	Reason
Coccinella 7-punctata	Aphidophagous, mainly on low growing plants
Adalia 2-punctata	Aphidophagous, habitat generalist
Adalia 10-punctata	Aphidophagous, deciduous tree specialist
Propylea 14-punctata	Aphidophagous, habitat generalist
Anatis ocellata	Aphidophagous, conifer specialist
Anisosticta 19-punctata	Aphidophagous, reed-bed specialist
Coccinella 5-punctata	Aphidophagous, unstable river shingle specialist, endangered
Chilocorus renipustulatus	Coccidophagous, deciduous tree specialist
Halyzia 16-guttata	Mycophagous, deciduous tree specialist
Thea 22-punctata	Mycophagous, mainly on low growing vegetation, particularly hogweed

Over the next year the survey team are hoping to form collaborations with other entomologists who have special interests in other groups that depend on aphids, such as Neuroptera, syrrphids and aphid parasitoids, to assess the effect of *H. axyridis* on other aphidophages.

The spread of *H. axyridis* in Britain

As a result of the media attention that the initial press release invoked, a number of records of *H. axyridis* taken in Britain before 19th September 2004 came to light. These included records of adults taken in moth traps, a photograph of a larva, and records of pupae and the subsequent adults found on imported flowers bought in a supermarket. These earlier records are valuable for two reasons. First, they increase the accuracy of the earliest record, moving the date back from September to July 2004. Second, they increase our confidence that *H. axyridis* only arrived in Britain in 2004, for all the records that came to light following the media attention on *H. axyridis* in October 2004 were of individuals recorded in that year (Majerus *et al*., in press a).

The distribution of *H. axyridis* in England is shown in Fig. 1. This figure differentiates between sightings made before 15th March 2005 and those made afterwards. The date of 15th March for the formal launch of the survey was not chance. It was chosen specifically to raise public awareness at the time when ladybirds would be becoming active and dispersing from their overwintering sites. Consequently, on the assumption that there is little or no dispersal by *H. axyridis* during the winter months, records made prior to 15th March 2005 effectively give the known distribution in the year of arrival. Records subsequent to 15th March may give an indication of how the species is spreading, although it is probable that some of the isolated records away from the main distribution are of additional importations from Europe or North America.

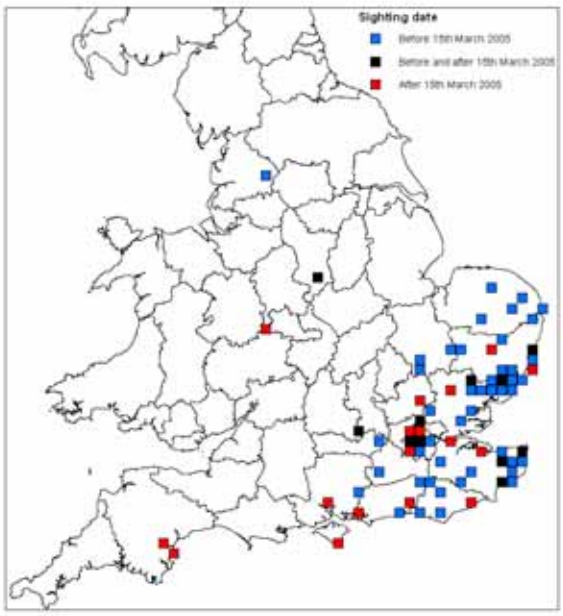

Figure 1: The distribution of *H. axyridis* in Britain. X indicates records received prior to 15th March 2005; O indicates records received since this date. Only verified records are included.

Potential areas of interest

The funded work at present focuses on monitoring the spread and impact of *H. axyridis*, developing online recording and appraising the methods used to monitor the ladybird so that they can be adapted as appropriate for other invasive species. However, the arrival of *H. axyridis* provides many other challenges and scientific opportunities.

Adaptation and selection

Harmonia axyridis is a very adaptable species. It has a wide natural latitudinal range. It occurs from central Russia in the north to China and the Himalayas in the south. Its range thereby extends from sub-tropical climes, through Mediterranean and temperate areas to sub-arctic and alpine regions. It has thus adapted to a variety of climates, from the intense continental climate of central Asia, with hot dry summers and very cold winters, to the humid, mild, maritime climate of western Japan.

Harmonia axyridis also has a diverse diet, most often feeding of aphids, but also being able to develop and reproduce on many other prey, such as coccids, adelgids, psyllids, and the immature stages of a variety of insects, including other Coleoptera, Lepidoptera, Diptera and Neuroptera (Hodek, 1996; Koch, 2003). In addition it will feed on pollen, nectar, honey-dew, fruit juice and tree sap, particularly just before and after periods of dormancy.

The recent arrival of *H. axyridis* in Britain provides an opportunity to investigate features of its adaptability and how natural selection affects it in its new environs. It is likely that the populations of *H. axyridis* now in Britain are derived from a relatively small number of invaders and that the variability within the gene pool of the British population is much less than in the gene pool of the species both globally and that in continental Europe: i.e. there will be some Founder Effects. This presents an opportunity to investigate how *H. axyridis* adapts to the conditions in Britain over the next few years. By sampling populations in Britain now, it should be possible to follow changes in a number of traits as the species is affected by selection. Several types of characteristic may be appropriate targets for this type of study.

Colour and pattern variation

Harmonia axyridis is a highly polymorphic species, showing great variation in the colour patterns of the elytra and pronotum. In its native range, the elytra vary from orange or red, with anything from 0 to 21 black spots (f. *succinea*). The spots are most commonly discrete, but in some individuals some or all of the spots may be fused. The nominate form, f. *axyridis*, which is the predominant form in much of Russia, has a black grid pattern on an orange or red background. In Korea and Japan f. *succinea* and two melanic forms, f. *conspicua* (black with two large red or orange spots), and f. *spectabilis* (black with four red or orange spots) are most common, and f. *axyridis* is rare. Other forms with large splashes or stripes of orange or red on a black background, or strong orange bars, with or without black spots in the bars, occur more rarely in parts of the native range.

The mode of inheritance of many of these forms have been elucidated, and in general the forms are controlled by various alleles of major genes (Hosina, 1933, 1936; Tan and Li, 1934; Komai, 1956).

In North America, f. *succinea* is by far the most common form, with few melanics having been recorded and f. *axyridis* absent. In Britain, only the forms *succinea*, *conspicua*, and *spectabilis* have been seen, with the overall frequencies of these forms from all individuals observed in 2004 being f. *succinea* = 0.557, f. *spectabilis* = 0.255 and f. *conspicua* = 0.188. The frequency of these forms and any additional forms found will be recorded annually at sites in south-east England, and elsewhere as the species spreads across Britain.

Melanic polymorphism in ladybirds has received considerable interest in the past (Majerus, 1998 for review), partly because the existence of genetic colour pattern polymorphisms in aposematic species is at odds with the rationale underlying the evolution of aposematic colour patterns and Müllerian mimicry (Joron and Mallett, 1998; Majerus, 2003). Despite many factors having been implicated in the evolution and/or maintenance of melanic polymorphism, no explanatory consensus has been reached. Monitoring the changes in melanic frequencies of *H. axyridis* in Britain over the next decade, as it adapts to British ecosystems, may provide invaluable data on the evolution and maintenance of melanic polymorphism in coccinellids, and the wider evolutionary conundrum posed by the existence colour pattern polymorphisms in aposematic species.

Life history traits

The life history of *H. axyridis* in Asia is well known. It will breed continuously in favourable conditions, having no requirement for diapause before maturing gonads as some coccinellids have. In consequence, it may produce anything from one to five generations per year (Wang, 1986; Sakurai *et al*., 1992; Osawa, 2000). In some parts of its range it has a dormant period during the hottest part of the summer when aphids are unavailable. Here, two generations, one in spring and one in autumn, are the norm. In late summer or autumn, adults fly from feeding

sites to overwintering sites, seeking shelter in rock crevices, often at altitude. Many of the beetles may find the same site; so aggregations running into hundreds, thousands or even tens of thousands may form.

In England, in 2004, *H. axyridis* was still ovipositing in September, with the result that larvae were still feeding in late October, long after native coccinellids had moved to overwintering sites. Due to lack of aphids and other food by late October, many of the larvae resorted to cannibalism, attacking larvae, pre-pupae and pupae. These observations suggest that the colonising *H. axyridis* are not precisely adapted to the maritime British climate.

Monitoring specific populations through the year, for a number of years, may provide valuable data on the evolution of life history traits. Such monitoring should be regular through the spring, summer and autumn, with the numbers of each life history stage being recorded. In addition, prey type and availability should be quantified (Sloggett and Majerus, 2000) and adult size (weight and pronotal width) should be recorded. Environmental measures, particularly temperature and sunshine hours can be obtained from local meteorological stations as necessary.

Biased sex ratios and male-killers

The sex ratio in most sexually reproducing animals is close to 1:1. This is adaptive, because it is usually selectively favourable to produce the rarer sex (Darwin, 1871; Fisher 1930). However, many populations of *H. axyridis* have a sex ratio that is biased in favour of females. This is because some females harbour a bacterium (a group VI *Spiroplasma*) that kills male hosts early in embryogenesis (Majerus *et al*. 1999). The rationale underlying this 'male-killing' is that the bacterium is maternally inherited and cannot be transmitted from male hosts (Majerus & Hurst, 1997). However, by killing male hosts at the egg stage, the bacterium makes the resources in the eggs available to sibling female larvae of the clutch. For neonate larvae, these extra resources provide a significant fitness increment, allowing the larvae longer in which to find their first live prey after dispersing from their egg batch (Hurst *et al*., 1992). The advantage from eating dead male eggs is gained by both the female ladybird larvae and by the bacteria they contain which are clonally identical to those that killed the male embryos. This then is a perfect example of kin selection, for the bacteria in males, by killing their hosts and effectively committing suicide in the process, give benefit to their clones in female hosts (see Majerus, 2003 for review).

The evolutionary dynamics of male-killing bacteria and other ultra-selfish genetic elements (so-called because their success depends on the harm they do to their hosts) have rarely been studied in the wild. In ladybirds, three factors – the vertical transmission efficiency of the bacterium, the direct fitness effect it has on infected females, and the benefits female progeny of infected females gain from the death of their brothers – are critical to the bacterium's population and evolutionary dynamics (Hurst *et al*., 1997). Field assessments of these parameters are difficult, particularly if the prevalence of the bacterium has reached a stable equilibrium prevalence in its host. However, it is unlikely that the prevalence of the male-killing *Spiroplasma* in the colonising populations of *H. axyridis* will be precisely equivalent to the stable equilibrium prevalence appropriate to conditions in Britain. The arrival of *H. axyridis* in England may thus provide a unique opportunity to study populations in which the prevalence of an ultra-selfish genetic element is changing under selection in the wild. Monitoring field prevalences of the bacterium and assessing the three critical parameter values both in the field and under controlled laboratory conditions should allow theoretical models of the dynamics of an ultra-selfish genetic element to be tested and the selective factors involved to be identified.

The control of *H. axyridis*

In Asia, North America and north-western Continental Europe, *H. axyridis* is one of the top predators within aphidophagous and coccidophagous guilds (see Koch, 2003, for review). Moreover, evidence is accumulating, particularly from North America, that shows that *H. axyridis* is having an adverse effect on native ladybirds, other aphidophages and on non-target prey species. Majerus *et al*. (in press b) have already predicted that the arrival of *H. axyridis* in Britain will have a radical impact on communities centred on aphids and coccids. This leads to the question of whether anything may be done to ameliorate this impact.

Use of insecticides to control *H. axyridis* numbers seems inappropriate because work on the effects of various insecticides has shown that *H. axyridis* that it is no more susceptible than other coccinellids. Insecticides would thus damage native beneficial species as much as *H. axyridis*. It is feasible that *H. axyridis* populations might be suppressed by trapping using target specific pheromones. This avenue is already being investigated.

An alternative strategy would be to consider the use of some of the enemies of *H. axyridis* that occur in its native range. It should be stressed, however, that introductions of Asian predators, parasitoids and/or pathogens of *H. axyridis* should not be undertaken until a full risk assessment has been undertaken. Possible agents include the male-killing *Spiroplasma* mentioned previously, a scuttle-fly (Diptera: Phoridae), several hymenopterous parasitoids and fungal pathogens. Full risk assessments on the potential impacts of such alien species are time-

consuming and costly. Furthermore, introducing alien species may be unnecessary if the predators parasitoids and pathogens that kill native ladybirds in Britain also kill *H. axyridis*.

The susceptibility of *H. axyridis* to a variety of organisms that cause coccinellid mortality in Britain should be assessed both in the laboratory and in the field. These organisms include two common species of scuttle-fly, *Phalacrotophora fasciata* and *Phalacrotophora berolinensis*, which attack the pupae of many British ladybirds, the wasp *Dinocampus coccinellae* that parasitises adults of most Coccinellinae, the sexually transmitted mite *Coccipolippus hippodamiae* that is a sexually transmitted parasite of *Coccinella magnifica* in Britain, and various other species in Continental Europe, and the soil fungus *Beauveria bassiana* which is pathogenic to most coccinelllids. Early field observations have shown that *D. coccinellae* does parasitise *H. axyridis* in Britain, and laboratory tests have shown that *B. bassiana* is pathogenic to *H. axyridis*. Further work on the control potential of these parasitoids, parasites and pathogens should be designed to provide assessments of the impact these organisms may have on *H. axyridis* compared to their impacts on native ladybirds.

Conclusion

The arrival of *H. axyridis* in Britain is likely to have a significant impact on the aphids and coccids that occur in Britain and the ecosystems that surround them. As studies of *H. axyridis* in Britain have been initiated so soon after the species became established, and before it has had much impact, we have a rare opportunity to monitor the spread and increase of this invasive species, assess its impact on such communities and investigate avenues by which its negative effects can be minimised. In addition we have the unique chance to address a suite of evolutionary questions on the operation of selection in the field.

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