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# A perspective on selective breeding to improve biological control agents

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The selection of desired wing phenotypes of *A. bipunctata* as described in this thesis, boils down to selective breeding. This is a historical method widely used in plant and animal breeding, including some insects (e.g. bees and silk moths). Nearly a century ago, Mally (1916) already suggested to use genetic selection to improve natural enemies for biological control. Natural enemies have now been commercially produced as biological control agents for about a century, and the production has been professionalized over the past decades (Van Lenteren 2003b). However, intrapopulation diversity is still understudied (Wajnberg 2004), and selective breeding is little applied in this field (Hoy 2002).

Nevertheless, there is ample variation between and within populations of natural enemies for traits likely to be important in biological control (e.g. Bakker et al. 1993; Hopper et al. 1993; Lozier et al. 2008; Nachappa et al. 2010; Tabone et al. 2010; Wajnberg 2010; Wajnberg et al. 2012). Selective breeding is, therefore, potentially a powerful tool to select new or improve existing natural enemies (Hoy 1986; Narang et al. 1993; Nunney 2003). It is especially suited for augmentative control in controlled and closed environments such as greenhouses, and especially for species which have extensively been studied for their ecology (Hoy 1986), such as some ladybird beetles, predatory mites, and parasitoids.

Indeed, several studies report successful artificial selection of desired traits. Examples include the resistance for chemical pesticides in predatory mites and parasitoid wasps (Hoy 1986; Rosenheim and Hoy 1988; Johnson and Tabashnik 1993), drought and temperature tolerance in predatory mites and entomopathogenic nematodes (Hoy 1985; Shapiro et al. 1997; Strauch et al. 2004; Salame et al. 2010; Anbesse et al. 2012), and sex ratio in parasitoids (Hoy and Cave 1986; Ode and Hardy 2008). Some of these selected lines have proven successful in biological control after release (Hoy 1986; Hiltpold et al. 2010). There are few examples of such strains that have become commercially available, including a few predatory mites with enhanced pesticide resistance (Hoy 2002), and others that lost diapausing after artificial selection on this trait (Van Houten et al. 1995). However, in most cases the efficacy of the selected strains in biological control was not tested in the field or greenhouse (Hoy 1985). Future research should accumulate evidence that the effectiveness in the field can indeed be increased by selective breeding.

## Previous objections to selective breeding

In the 1980s, "genetic improvement" of natural enemies was debated. Genetic improvement covers selective breeding but also hybridization and genetic engineering (the latter two lie outside the scope of this thesis). In a review, Hoy (1986) has listed a number of reasons why she thinks producers of natural enemies have been slow in picking up genetic improvement tools for improvement of their products: (1) the fear that laboratory adaptation would erode genetic diversity and lead to decreased fitness in the field; (2) a lack of genetic knowledge, and (3) a negative image due to some previous failures.  She also mentions the high costs involved in this procedure (4), because of the labour-intensive development. I think that by now, the objections can be mostly overcome.

For example, the loss of genetic variation (objection 1) is a general risk for captive populations (Mackauer 1976), and there are several ways to reduce the loss of genetic diversity. These include starting with a large population, keeping high numbers during breeding, outcrossing events, hybridization of strains, and crossing inbred lines (Wajnberg 1991; Bartlett 1993; Hoekstra 2003; Nunney 2003). If genetic erosion would result in lower fitness of the natural enemies, this will be detected sooner when recently developed quality control guidelines  are applied (Leppla 2003; Van Lenteren et al. 2003). These guidelines include the regular measurement of many fitness components. It has even been suggested to develop “quality assurance” rather than “quality control” in biological control, which should guarantee the quality of commercially produced natural enemies rather than detect qualitative flaws that need to be corrected for (Bolckmans 2003). This trend should reduce the fear of genetic erosion by artificial selection (objection 1).

The recent advantages in the field of genetics and genomics will also render the process of selective breeding less labour intensive. Molecular tools can help to unravel the genetic architecture of traits (objection 2), including the identification of loci regulating these traits. Recently, genetic regions underlying several traits with relevance to biological control have been identified in natural enemies, including parasitoid host preference (Desjardins et al., 2010), clutch size, and sex ratio (Pannebakker et al. 2011). The latest developments in genomic and sequencing technology, will render the localization of the genetic basis of such traits more affordable and common practice. Once loci have been identified, molecular markers can be developed to facilitate screening of traits lacking morphological phenotypes. This can be both used in an inventory of the natural variation for these traits in the field, and of the individuals used in the breeding program (“marker-assisted selection”)(Ribaut and Hoisington 1998; Dekkers and Hospital 2002). Especially for life-history or behavioural traits, this could save a lot of time (objection 4).

I am not aware of the current image of “genetic improvement” (objection 3), but I think that exploiting natural variation by selective breeding will be more easily accepted than genetic engineering by the public, and the natural character of the method can even be used in promotion. Biocontrol researchers might also need to put more effort in to promoting their positive results (Van Lenteren 2012).

Finally, when the benefit gained from the enhanced phenotype is large enough, this can outweigh the costs (objection 4). Cost-benefit analyses are scarce (Hoy 2002), but one of the studies estimated that the production costs of an egg parasitoid are reduced to a third when the sex ratio is modified in favour of the number of females (Irvin and Hoddle 2006).

## Why is selective breeding timely?

Although the potential for selective breeding, or more generally, genetic improvement, of natural enemies has been highlighted in the past (Hoy 1986; Hopper et al. 1993; Narang et al. 1993), it is still hardly exploited in the development of biological control agents (Hoy 2002). I think there are several reasons why this method currently deserves a boost.

First, the practice of biological or integrated control of arthropod pests in agriculture is growing, and so is the number of commercially available natural enemies (Van Lenteren 2003c, 2012). This trend is likely to continue with increasing legal limitations on the use of pesticides. However, there are still pests that are currently not sufficiently controlled, including emerging pest species. New biological control agents should be developed, or existing species improved, that match with current integrated pest management strategies to control these pests. In recent decades, the commercial production of natural enemies has been professionalized (Van Lenteren 2012), and the field of genetics and genomics has made considerable progress. Altogether, there is more money, knowledge, and market for the implementation of new methods in the development or improvement of natural enemies for augmentative biological control.

Second, natural enemies are traditionally selected from the natural environment of the insect pest. As many insect pests originate from other countries, this frequently involves the import of non-native insect material which can involve risks for local biodiversity (Roy and Wajnberg 2008; De Clercq et al. 2011). In addition, recent international regulations impede the use of exotic species for biological control. The Convention on Biological Diversity (see www.cbd.int) hampers the export of natural enemies for biological control (Cock et al. 2010; Van Lenteren et al. 2011), whereas the FAO guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms demands a critical evaluation of species imported (Secretariat of the International Plant Protection Convention, 2005). These developments are likely to increase the need to explore and exploit natural variation in native natural enemies. There is already a trend towards preferentially utilizing indigenous natural enemies, with the indigenous natural enemies introduced to the European market outnumbering the exotic ones this century, whereas a reverse pattern was observed for the past century (Van Lenteren 2012).

## How to exploit natural variation?

While very promising, selective breeding of biological control agents should only be used when no better naturally adepted species is available and safe for biological control. Therefore, both interspecific and intraspecific genetic variation should be evaluated when developing natural enemies for biological control. When a species meets the requirements for biological control, this should be favoured over selective breeding of another sub-optimal species. For example, a strain of the parasitoid wasp *Aphitis lignanensis* Compere tolerant to extreme temperatures was developed (White et al. 1970) for areas of California with such climate, but was never applied because the species *Aphitis melinus* DeBach that was naturally adapted to such climate was established in the area (Nunney 2003).

Improving natural enemies for biological control by means of selective breeding requires a good characterization of intraspecific genetic diversity for the traits of interest (Narang et al. 1993; Wajnberg 2010). Traditionally, populations from different geographical locations are compared (Wajnberg 2004). Although these may differ strongly in traits, these populations do not always represent 'evolutionary significant units'. For example, Lozier et al. (2008) found that genetic distance did not correspond very well to geographical distance, and that large genetic variation existed within geographical areas in a parasitoid wasp. In another parasitoid wasp, Vink et al. (2012) showed that genetic variation was closely associated with the host taxon, but not to geographical location. It is, therefore, recommended to collect large numbers of specimens from several locations and host plants, to capture the majority of genetic variation in the wild as the basis for selection. Then, intra-population heritability of traits should be assessed to predict the response to selection (Falconer and Mackay 1996; Wajnberg 2004). When selective breeding succeeds in improving the trait of interest, it should be tested whether this is indeed translated into improved mass-rearing or biological control efficacy.

## Case study of *Adalia bipunctata*

In this thesis, I have shown that aphid control by *A. bipunctata* can be improved by artificial selection of a naturally occurring wingless genotype (chapter 2), and that selection on the expression of this trait (chapter 5) may enhance its suitability for mass-rearing (chapter 8). Variation in wing length is, however, not the best example to generalise the potential usefulness of intra-population diversity for biological control agents, because the wingless phenotype seems to be unique and appears not to be adaptive (chapter 8). However, variation in many other traits is adaptive and more common, and moreover is potentially of interest for biological control.

For example, colour polymorphism is common in *A. bipunctata*, where dark (melanised) and red (typical) morphs coexist within populations (Brakefield 1984a). Because of the thermal properties of the melanisation, melanic morphs heat up in solar radiation earlier and are more active than typica morphs, at cool temperatures (De Jong et al. 1996). Therefore, melanic morphs might be more effective in biological control in areas with lower temperatures, such as greenhouses with lower temperatures, and outdoors in cool climates (chapter 3). Since colour polymorphism is entirely under genetic control in this species (Majerus 1994), it can easily be fixed in mass-reared populations.

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