Feature

The benefits of bottlenecks

Population bottlenecks are commonly thought to be disadvantageous because they deplete genetic variation. But they can be advantageous too, in particular for biological invaders like the harlequin ladybird. **Florian Maderspacher** reports.

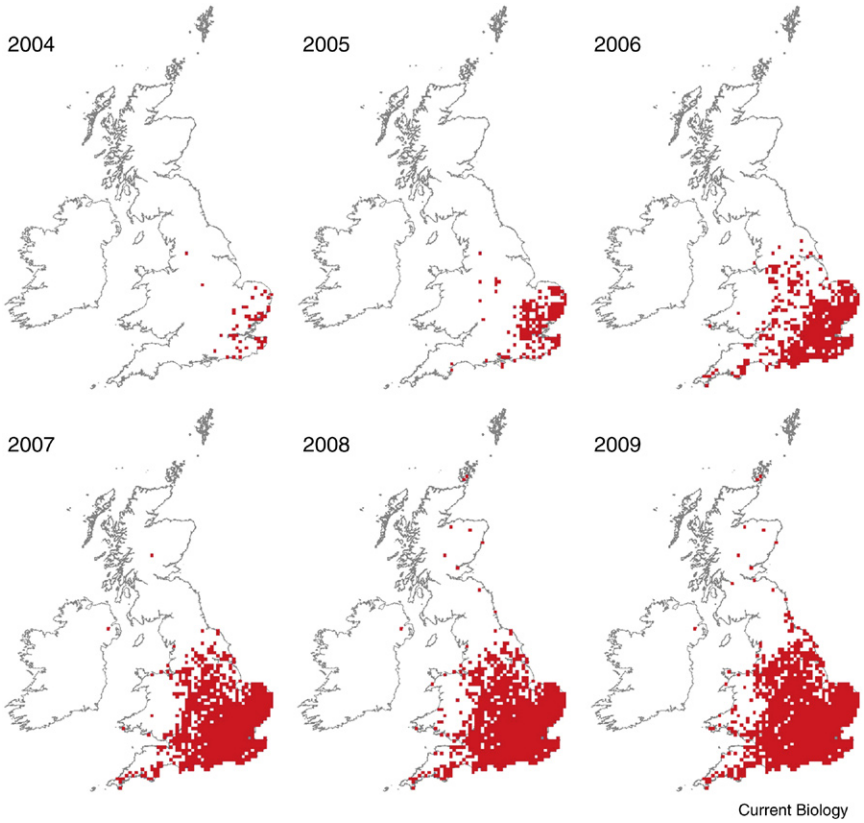
The last successful invasion of Britain began in the summer of 2004. Like all evils, it stemmed from the continent, and quickly the invaders were rumoured to be smelly, to squat in houses by the thousands and to damage the livelihood of their autochthonous relatives. The invaders go by the harmless-sounding name of *Harmonia axyridis* or harlequin ladybird, but there is nothing harmonious or lady-like about them. These beetles spread at a fierce pace and are more ferocious eaters and breeders than other ladybird species. In autumn, they can form spectacular mass aggregates, which may have contributed to their notoriety. Like other famous examples of biological invasions — grey squirrels in Britain, rabbits in Australia or giant hogweed in central Europe — their success is staggering, which for biologists begs the question why this should be so.

Biological invaders do suffer one obvious disadvantage, namely that at the beginning of the invasion their numbers are low. Such so-called population bottlenecks mean that the invader populations can go extinct quite easily, and that, compared with larger native populations, their genetic variability will be lower. But this need not always be a disadvantage, as a paper by Benoit Facon and colleagues on harlequin ladybirds in this issue of *Current Biology* illustrates. In fact, a bottleneck of the right size can have quite the opposite effect — it can purge a population of deleterious alleles and thus increase their fitness rather than reduce it though inbreeding depression.

Harlequin ladybirds stem naturally from Central and Eastern Asia. Unlike many other species of the ladybird family (Coccinellidae) that can be identified by the number of spots on their hardened wings, these beetles' colour patterns are highly variable. Harlequin ladybirds are also bigger than most of their relatives and are voracious aphid eaters. Their appetite and their prolificacy make them a potential threat to resident plant and animal species. Although the actual impact is as yet unclear, Peter Brown from the UK ladybird survey notes that "there is growing evidence that native ladybirds — and some other insects — are being negatively affected, probably due to direct predation, but also due to competition for shared resources. Most at risk are species with a high niche overlap with the harlequin, in particular, the two-spot ladybird (*Adalia bipunctata*)."

Ironically, their big appetite was precisely the reason they were introduced to the US as early as 1916 to protect crops against aphids and other pests. However, it was not until 1988 that a stable population had established itself in Louisiana. Within the next 20 years harlequin ladybirds spread rapidly within the US and Canada, but also into Europe and South America — where there had been previous small-scale introductions — and even to South Africa. The long lag phase between initial colonisation and full-fledged invasion is quite typical for biological invasions. Recent genetic analyses of several invasive ladybird populations across the world indicated that they all are derived from the American population. In the militaristic language used by students of biological invasions, such a population is called a 'bridgehead'. But what could have happened to the bridgehead that made the ladybird invasion all of a sudden so virulent?

Purged: Invasive harlequin ladybirds showing their characteristic extreme variability in colour patterns. (Photograph: William Mettey.)



Beetle of Britain: Spread of the harlequin ladybird in Britain between 2004 and 2009. Images courtesy of CEH/UK Ladybird Survey.

Intuitively, new invaders are almost always at a disadvantage. They are usually low in numbers and generally less well adapted to the new environment than the autochthons. But of course, organisms can evolve and adapt to new conditions. Yet, as invader numbers are low, they also bring with them fewer genetic variants than the source population. Such a genetic bottleneck means that there is less genetic raw material for selection to act upon. And thus, invaders might not be able to adapt quickly to their new environment. In addition, the genetic bottleneck can lead to inbreeding depression — the reduction of the population's fitness due to an increased likelihood of breeding between relatives that share deleterious genetic variants. This is a somewhat paradoxical situation: population genetic theory predicts that genetics should act against invaders, yet sometimes they flourish nonetheless.

One possible way out of the bottleneck conundrum is that, in terms of genetic diversity, there is actually no bottleneck. Indeed, studies on several invasive species have shown invaders can have quite high genetic variability. Brown anole lizards, for instance, came to Florida first in the late 19th century and began to spread — after the usual lag phase — in the second half of the 20th century. An analysis of the invaders' population genetic make-up, published in 2004 by Jason Kolbe, Jonathan Losos and others (Nature *431*, 177–181), indicated that variation was unexpectedly high. As Jason Kolbe says, they were "quite surprised at the overall pattern of high genetic variation; conventional wisdom would have suggested one or a few native-range populations as the source of the invasion." Instead, there must have been at least eight different introduction events, from different source populations. That way, the invader population becomes actually highly diverse — a sort of nutshell version of the variation of several original populations. This highly concentrated genetic variation might be one foundation of their high invasive potential. Jason Kolbe notes: "If the elevated level of variation I found for neutral markers is indicative of adaptive variation, then rates of adaptive evolution may be enhanced." Notably, in line with the bridgehead scenario, anole lizards of Florida then went on to invade places further afar, such as Taiwan and Hawaii.

That increased genetic variance accrued through multiple invasions can be beneficial for the invader was substantiated through a 2007 study by Benoit Facon and colleagues (Curr. Biol. *18*, 363–367) on the freshwater snail *Melanoides tuberculata*, which has invaded the Caribbean island of Martinique. (Biological invaders, or those who study them, do seem to have a penchant for scenic locales.) *M. tuberculata* invaders exhibit a much higher genetic variability than any of their multiple source populations. But, the invaders also show extremely high variation in important fitness-related traits such as fecundity and size. So again, rather than a bottleneck that depletes variation, invasive populations can be like a funnel that bundles and concentrates variation.

But for the harlequin ladybirds, this does not seem to apply. For their new study, Benoit Facon and his colleagues chose the harlequin ladybird as a model, as it "has invaded several different geographical areas, which represents replicate introductions, and is a good example of invasion after a substantial lag time", Facon explains. And indeed, when they compared invasive and native populations, Facon and his colleagues found that there is indeed evidence for a genetic bottleneck with reduced genetic variation.

Population genetic theory had long suggested that a bottleneck need not be entirely bad. Because of the smaller number of breeding individuals, after some generations, the likelihood of siblings mating will increase during the bottleneck. This means inevitably that the overall level of homozygosity in the population will increase. When an allele is recessive and has a deleterious effect, or is even lethal, the presence of more homozygotes will mean that this allele declines in frequency. In other words, the bottleneck population will be effectively purged of such deleterious alleles.

Theoretical studies have shown that the effectiveness of purging depends on several variables, such as the size of the population, the strength of the allele's negative effects, the strength of selection and the duration of inbreeding. And indeed, the duration and size Facon and colleagues modelled for the invasive ladybirds' bottleneck — 20 generations and <10% of what would be considered a healthy

population size — seemed to fit well the predictions for purging to occur.

But did purging actually take place? In the next step, the authors measured the extent of inbreeding depression in native and invasive species. Beetles from three native and three invasive populations were mated either with their siblings (inbreeding) or with unrelated individuals (outbreeding), and fitness-related traits were compared. Both in terms of generation time and reproductive output, the invasive populations performed significantly better than the native populations.

"Inbred individuals of invasive populations are clearly fitter than inbred individuals of native populations and as fit as outbred individuals from both types of populations," explains Benoit Facon, and "this means that this decrease of inbreeding depression in invasive populations is due to a loss of deleterious mutations, namely purging."

Even though the notion of purging in small populations has been studied intensely on theoretical grounds, empirical evidence had been scarce. So far, the effect had mainly been shown in laboratory animals, like fruit flies. "The potential effects of population bottlenecks during invasion are mixed, in some cases enhancing additive genetic variance and in other cases decreasing variation, so it's great to see such clear-cut empirical evidence for their role", says Jason Kolbe, and "the combination of molecular markers, simulations and breeding experiments to measure fitness-related differences between introduced and native populations makes this study unique."

Of course, it is as yet not clear if the observed purging effect is really responsible for the ladybird invasions. And it is also not entirely certain that purging has not occurred before invasion in one of the founding populations. But it is certainly tempting to speculate that such purging effects might contribute to the striking success of the harlequin ladybirds and possibly other invasive species. In the words of Jason Kolbe: "population bottlenecks may be a case of 'damned if you do, damned if you don't' for invasion success." And who knows, perhaps native species that might suffer from the invasive harlequin ladybirds can one day bounce back having slipped through their very own purging bottlenecks.

Quick guide

Drosophila embryonic hemocytes

Iwan Robert Evans and Will Wood*

What are they? *Drosophila* embryonic hemocytes are the highly motile macrophages that represent the main cellular arm of the innate immune system in this organism. These cells are specified during embryonic development and persist through larval stages to adulthood.

Any pseudonyms? Also known as haemocytes, plasmatocytes (this is more specific since strictly speaking the hemocyte lineage includes lamellocytes and crystal cells as well as plasmatocytes), or *Drosophila* macrophages, phagocytes or blood cells.

Where do they come from? There are two waves of hemocyte production; the first occurs in the head mesoderm, while the second occurs in a stem cell niche in the lymph gland. Early hemocytes are speculated to be equivalent to primitive embryonic blood cells and disperse to cover the entire embryo, whereas lymph gland hemocytes are released during late larval stages and in response to parasitisation. Homologs of the GATA (Serpent) and Runx (Lozenge) families of transcription factors involved in vertebrate hematopoiesis play important roles in hemocyte specification, whilst hemocyte plasma membranes are packed with molecules related to those found on vertebrate macrophages (e.g. Croquemort, a CD36 homolog, and Draper and Nimrod, scavenger receptors that resemble CED-1-like proteins such as MEGF10 and Jedi in vertebrates).

Where do they go? Hemocytes migrate out from the head along two main pathways to disperse over the entire embryo: dorsal migration, involving penetration of an epithelial barrier to enter the extended germband, which carries them posteriorly during germband retraction, and ventral migration along the ventral nerve cord (Figure 1A). The two populations meet on the ventral nerve cord and then migrate laterally, ensuring an even spread over the embryo.

And what do they do? Hemocytes are important in both development and immunity. Without hemocyte function embryos fail to develop correctly, with defects in the ventral nerve cord due to the roles of hemocytes in uptake of apoptotic corpses and possibly also secretion of matrix, since these cells are responsible for the secretion of much of the extracellular matrix and express numerous matrix-remodelling enzymes. Hemocyte-derived matrix also potentiates bone morphogenetic protein (BMP) signalling in the developing renal tubules and hemocytes are therefore required for the correct morphogenesis of these structures. Hemocytes are also able to recognise and respond to pathogens and epithelial wounds at both embryonic and larval stages. Although hemocytes are dispensable for wound closure, they are necessary for protection against infection. In fact, although hemocytes are essential to complete embryogenesis due to their developmental roles, during larval stages their primary role appears to be the phagocytosis of pathogens because larvae that lack hemocytes can only survive through to adulthood if reared under sterile conditions.

What gets them going? Hemocytes appear to disperse primarily in response to the expression of platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF)-related ligands (Pvfs) that are expressed along their route ways in the embryo, but restriction of space also plays a role in constraining where they can migrate. Other ligands controlling these migrations remain obscure, although cell–cell repulsion, a process that requires the microtubule-binding protein Orbit/CLASP, may contribute to their dispersal and/or maintenance of their even distribution in the embryo. The open circulation system in *Drosophila* larvae and adults means that hemocytes are passively pumped around the hemolymph by the dorsal vessel (the heart equivalent); this difference means that, unlike in