that approximately 80% of the landscape is invaded by earthworms to some degree. However, only about 50% of invaded sites contain more than four earthworm species, and sites with only one or two species present appear to have relatively minor ecological impacts. In contrast, in the boreal forest of Alberta where Cameron *et al*. (2008) conducted their study, they estimate that only 9% of forest habitat is likely invaded due to the more recent history of human development. Moreover, threats posed by earthworm species just beginning to expand across North America, such as the Asian genera *Amynthas*, are not yet fully appreciated (Kourtev *et al*. 1999; Hale 2007). Areas likely to have multiple, ongoing introductions would be places where decisive action now could mitigate against the introduction of non-native earthworms species still uncommon in most of North America yet are known to cause severe impacts outside of their native ranges. Therefore, even in areas that have been invaded to some degree by earthworms, efforts to prevent future introductions of new species or additional genetic variability of species already present could help to limit the level of impacts.

Cameron *et al*. (2008) demonstrate how the use of relatively simple and inexpensive molecular techniques developed in recent years can provide tools to address otherwise difficult questions. Molecular biology can now play a powerful role in the battle against non-native species by illuminating many of the patterns of introduction and spread that are otherwise difficult or impossible to ascertain. This study is the first to directly determine that human-mediated dispersal is much more important to the continued expansion of non-native earthworm species than is natural spread from diffusion of existing populations. Further, the fact that multiple introductions appear to be clustered at sites like boat landings provides clear support for the idea that changes in human behaviour can have pivotal affects on the future trajectory of these invasions. The prevention of future introduction is key to the protection of northern forests and the resources they provide, and this study will no doubt lead to more effective targeting of limited resources in education and research as well as inform potential regulatory or policy decisions.

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Can we stop transgenes from taking a walk on the wild side?

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Abstract

Whether the potential costs associated with broad-scale use of genetically modified organisms (GMOs) outweigh possible benefits is highly contentious, including within the scientific community. Even among those generally in favour of commercialization of GM crops, there is nonetheless broad recognition that transgene escape into the wild should be minimized. But is it possible to achieve containment of engineered genetic elements in the context of large scale agricultural production? In a previous study, Warwick *et al*. (2003) documented transgene escape via gene flow from herbicide resistant (HR) canola (*Brassica napus*) into neighbouring weedy *B. rapa* populations (Fig. 1) in two agricultural fields in Quebec, Canada. In a follow-up study in this issue of Molecular Ecology, Warwick *et al*. (2008) show that the transgene has persisted and spread within the weedy population in the absence of selection for herbicide resistance. Certainly a trait like herbicide resistance is expected to spread when selected through the use of the herbicide, despite potentially negative epistatic effects on fitness. However, Warwick *et al*.'s findings suggest that direct selection favouring the transgene is not required for its persistence. So is there any hope of preventing transgene escape into the wild?

Keywords: *Brassica napus*, *Bo rapa*, gene flow, hybridization, transgene

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Fig. 1 Weedy *Brassica rapa* (orange flags) growing in a *B. napus* field. (Photo: MJ Simard)

Transgenes have the potential to improve crop nutrition, increase yield, reduce inputs of chemical herbicides and insecticides, and lower fuel use by farmers (Baenziger *et al.* 2006; Brookes & Barfoot 2006). These benefits are not trivial; for example, the use of herbicide resistant genetically modified (GM) canola has reduced herbicide applications by an estimated 22% and increased farm income by ca. 10% in North America over the last 10 years (Brookes & Barfoot 2006). Nevertheless, transgenes introduce novel traits with unknown consequences for both the consumer and the environment (Gillett *et al.* 1986). Indeed, transgenic crops are employed precisely because they possess traits that do not exist in the gene pools of crops and their close relatives.

One of the concerns about the use of transgenes noted most often is the potential for transgenes to spread from crops into natural populations. We know that many plant species can be promiscuous—pollen shed on the wind may indiscriminately fertilize eggs of close relatives (Arnold 1992). For many years, this has led to efforts to minimize the inadvertent contamination of crops by pollen or seeds of wild relatives (or other cultivars) with less desirable traits. Concerns about gene flow in the other direction, from crops into the wild, have escalated with the widespread deployment of genetically modified organisms (GMOs) (Ellstrand *et al.* 1999). Many GM traits are likely to be favoured in natural populations, where they may alter

the abundance and distribution of crop relatives (Pilson & Prendeville 2004). For example, herbicide resistance would allow weeds to colonize transgenic fields (defeating the purpose of the transgene), and both disease and insect resistance might allow weeds to escape from their natural enemies, sparking invasion of both agricultural and natural areas (Keane & Crawley 2002; Pilson & Prendeville 2004). Risk management in the GM era is now focused on eliminating this gene flow. This task is far more challenging because the ultimate aim is to prevent (not minimize) transgene escape into the wild.

Warwick *et al.* (2003) had previously documented hybridization between transgenic canola, *Brassica napus*, and its wild relative *B. rapa* at two study sites in Quebec, Canada. At one site, some plants produced in excess of 50% hybrid progeny. In their latest study (Warwick *et al.* 2008), they demonstrate that hybrid lineages generated in 2000/2001 persisted for the 3–5 years of study at each site. They assessed evidence for hybridization and introgression using species-specific molecular markers [amplified fragment length polymorphism markers (AFLPs)], a transgene marker (herbicide resistance), and ploidy (*B. napus* is tetraploid, while *B. rapa* is diploid). They found that, while hybrid lineages declined dramatically over time, *B. napus* markers persisted in weedy *B. rapa*. One individual in 2005 displayed herbicide resistance in a *B. rapa* diploid genetic background. Although it suffered from reduced pollen fertility, this individual was still able to produce hundreds of offspring, most of which showed high pollen fertility and half of which showed herbicide resistance. Importantly, this transgene persisted in the weedy *B. rapa* populations despite a lack of selection by the appropriate herbicide after 2002.

Two key points emerge from these results: (i) introgression occurs despite genetic barriers; and (ii) transgenes can persist in the environment without the aid of strong selection. Matings between diploids and tetraploids typically produce triploids with low fertility (e.g. Burton & Husband 2000), and indeed this is the case in *B. rapa* × *B. napus* (Warwick *et al.* 2003). This barrier is likely to be critical for maintaining species boundaries. However, low fertility is not equivalent to sterility, permitting the type of introgression seen in this study. Crops and their weedy relatives frequently co-occur over North America's vast agricultural areas, creating ample opportunities for these rare events (Ellstrand *et al.* 1999). In this case, weedy *B. rapa* is relatively uncommon in eastern North America, but it does occur in *B. napus* growing areas (Simard *et al.* 2006). If *B. rapa* occurs at low densities near canola fields this may in fact enhance rates of gene flow from the crop; because *B. rapa* is self-incompatible, the absence of compatible conspecific mates may favour successful pollination by *B. napus*. Given that partially fertile hybrids are produced, the likelihood of persistence of individual traits will then depend on their genetic architecture and fitness effects. Simple genetic constructs such as the herbicide resistance transgene in this canola, which appears to carry no inherent fitness cost in the genetic background of *B. rapa* (Warwick *et al.* 2008), may persist but are unlikely to become widespread following single bouts of gene flow. However, if hybridization occurs repeatedly across many populations or the trait is selectively favoured (e.g. through the use of the herbicide), its spread is virtually assured.

For transgenic crop production, such news is sobering. Evidence is mounting that crop × weed hybridization is the rule rather than the exception for some crops and that transgenes regularly escape (Ellstrand *et al.* 1999; Pilson & Prendeville 2004; Andow & Zwahlen 2006). Transgenes that confer a direct fitness cost to weedy species, or that can be tightly linked to such a disadvantageous gene, might hold the most promise for 'safe' deployment (Andow & Zwahlen 2006). However, simulation studies suggest that these improved mechanisms will likely only slow transgene escape, and containment is likely to be achieved only if the rate of escape becomes low relative to the expected market lifespan of a cultivar (Haygood *et al.* 2004). Furthermore, fitness costs change with the genetic and ecological environment, and it will be hard to ensure that even an apparently disadvantageous crop gene will not be favoured or neutral in some weedy populations (Bergelson & Purrington 1996). Indeed, introgression is a key source of variation in the evolutionary history of plants (Arnold 1992); ironically this may prove to be the most natural feature of transgenic crops.

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