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Published in:
Journal of Applied Ecology

DOI:
[10.1111/j.1365-2664.2008.01508.x](https://doi.org/10.1111/j.1365-2664.2008.01508.x)

[Link to publication](#)

Citation for published version (APA):
Hooftman, D. A. P., Oostermeijer, J. G. B., Marquard, E., & den Nijs, J. C. M. (2008). Modelling the consequences of crop-wild relative gene flow: A sensitivity analysis of the effects of outcrossing rates and hybrid vigour breakdown in *Lactuca*. *Journal of Applied Ecology*, 45(4), 1094-1103. <https://doi.org/10.1111/j.1365-2664.2008.01508.x>

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Modelling the consequences of crop–wild relative gene flow: a sensitivity analysis of the effects of outcrossing rates and hybrid vigour breakdown in *Lactuca*

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Summary

1. Hybridization between crops and their wild relatives may enhance invasiveness and change their niche dynamics. This is regarded as a major biosafety problem in terms of the development of noxious, invasive weeds and the loss of the genetic identity of native species. Modelling the consequences of hybridization is becoming an important tool for risk assessment.
2. We conducted a sensitivity analysis of a stochastic hybridization model, predicting changes in genotypic population composition. The model includes various classes of hybrids between *Lactuca sativa* (lettuce) and its wild relative *L. serriola*, and is based on empirical demographic measurements of fitness (λ).
3. We calculated the sensitivity of these transitions and of the following model parameters: outcrossing rate, the temporal frequency of crop presence, early hybrid fitness, hybrid vigour breakdown rate and assumed fitness of advanced generation hybrids.
4. In a non-stochastic simulation, the wild relative was displaced by more vigorous hybrids. The relative fitness of late generation hybrids in relation to the fitness of the wild taxon had the strongest effects on the population composition in the long term.
5. The outcrossing rate affected the estimated population composition strongly but the proportional impact of this parameter was low compared to the effect of hybrid fitness. Moreover, the stochastic simulations showed that the level of stochasticity had only a small effect on the sensitivity of population growth rates to changes in any of the model parameters, except for changes in the fitness of the wild taxon.
6. *Synthesis and applications.* It is essential to determine the relative fitness level of advanced generation hybrids, as this has a much stronger proportional effect than other factors. Future risk assessment should focus more on long-term fitness effects and not only on the outcrossing rate and the early establishment of hybrids. Experiments with multiple generations and analysis of hybrid vigour in modelling efforts would yield better predictions of which traits would be likely to introgress, and at what speed. This would be of benefit in the decision-making process and in future monitoring after crop release, for example of transgenes.

Key-words: biosafety; crop–wild relative gene flow, demography, fitness, genetic modifications, hybrid vigour breakdown, hybridization, *Lactuca*, lambda (λ), risk assessment

Introduction

Hybridization between crops and their wild relatives may enhance invasiveness and niche expansion of plant popula-

tions (Pilson & Prendeville 2004) and is therefore regarded as a major biosafety problem in terms of the development of noxious, invasive weeds and the loss of the genetic identity of native species (Gray 2004). In particular, the possibility that transgenes ‘escape’ from genetically modified crops into surrounding wild relative populations and their potential to equip the recipient populations with novel traits that make them invasive has been discussed extensively (Ellstrand 2003;

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Gray 2004; Snow *et al.* 2005; Andow & Zwahlen 2006). Experimental studies have repeatedly demonstrated that gene flow between crops and wild relatives may produce hybrids that are sufficiently fit to allow the subsequent introgression of crop genes into the genome of the wild population (Ellstrand 2003; Hails & Morley 2005; Andow & Zwahlen 2006; Chapman & Burke 2006). Successful and stable introgression of escaped crop genes, using field-based molecular observations, has already been shown for various species (e.g. Mikkelsen, Andersen & Jørgensen 1996; Whitton *et al.* 1997; Warwick *et al.* 2008).

From a management perspective, gaining insight into the long-term consequences of gene flow between crops and wild relative species is important. For this purpose, a modelling approach seems most appropriate because it allows the incorporation of stochastic variation and an extrapolation of the results from experimental studies to larger time-scales (Gray 2004; Snow *et al.* 2005; Allainguillaume *et al.* 2006). In particular, a sensitivity analysis may help to identify which parameters influencing a crop–wild relative interaction mainly determine the success of putatively emerging hybrids. This contributes to the development of more accurate and efficient risk assessment studies (Snow *et al.* 2005; Andow & Zwahlen 2006). Recent examples of such analyses are Colbach, Molinari & Clermont-Dauphin (2004), Claessen *et al.* (2005) and Garnier & Lecomte (2006).

A specific new (trans-) gene providing a continuous fitness increase to a hybrid population may either provide this in certain habitats (e.g. abiotic stress tolerance, Lexer *et al.* 2003) or, through changing life-history traits, increase vital rates (e.g. Hooftman *et al.* 2005; Campbell, Snow & Ridley 2006; Mercer, Shaw & Wyse 2006). Hence, the fitness of more advanced not heterosic hybrid generations could be increased above that of the wild relative taxon. Assumptions about the fitness of advanced hybrid generations are therefore crucial to include in modelling efforts.

Hooftman *et al.* (2007) developed a model to estimate the consequences of introgression of crop genes into a wild relative background genome over multiple generations based on empirical data. In this model, finite rates of population growth (λ) and all possible genotype transitions between parental and hybrid classes were combined with a wide range of outcrossing rates. The model predicted the formation and potential establishment of introgressant (sub-) populations. An important feature of the empirical data and the model was the inclusion of an estimate of the rate of hybrid vigour breakdown. Such a breakdown is caused by segregation of the initial heterosic loci or breakdown of positive epistatic loci. This genetic process is well-documented theoretically (Burke & Arnold 2001) and experiments have demonstrated that the performance and phenotypes of advanced generations of (backcrossed) hybrids become more similar to the parental taxa (Arnold & Hodges 1995; Gueritain *et al.* 2002; Hooftman *et al.* 2005; Johansen-Morris & Latta 2006, but see Campbell, Galen & Wu 2005). However, there is a lack of empirically and quantitatively determined levels of hybrid vigour breakdown in crop–wild relative hybrids. The same

holds for sensitivity analyses of hybridization models to variation in such rates.

In this study, we present a sensitivity analysis of the various parameters used by Hooftman *et al.* (2007) to model the long-term consequences of crop–wild relative gene flow in lettuce. Parameters include the outcrossing rate, factors concerning hybrid vigour breakdown and the initial fitness of different plant classes. Environmental stochasticity is an important feature of this model, since *Lactuca serriola*, as a typical ruderal species, occurs in a highly dynamic habitat. An application of our results is to improve targeting of future risk assessment strategies for crop–wild relative hybridization, with special focus on the release of transgenes.

Material and methods

SPECIES AND HYBRIDIZATIONS

For extensive descriptions of the two studied taxa, we refer to Tutin *et al.* (1976). In short, *L. serriola* L. (Asteraceae, $2n = 18$) is a common annual weed found in anthropogenically disturbed habitats throughout Europe, Northern Africa and North America. Plants are predominantly autogamous, with an average 1–5% outcrossing via insect pollen vectors; although individual plants may experience outcrossing rates as high as 20% (D'Andrea 2006).

Lactuca sativa L. (Asteraceae, lettuce, $2n = 18$) is a common annual crop, which flowers frequently in non-commercial private vegetable gardens, simultaneously and often sympatric with *L. serriola*. It is considered conspecific with, and derived from, *L. serriola*. Both taxa are fully interfertile, with no known pre- or post-zygotic barriers (Koopman, Zevenbergen & van der Berg 2001).

For details about the crossing scheme, the different crossing lineages involved, and the experimental set-up, as well as the results of prior experiments, we refer to Hooftman *et al.* (2005, 2007). Hooftman and co-workers presented empirical data on the fitness of parental species and four generations of hybrid classes, including the different hybrid vigour breakdown rates depending on the pathway by which the respective hybrids were produced. Hybrid classes were defined by their theoretically expected proportion of the *L. sativa* genome.

THE MODEL

Classes included in the analysis

For the purpose of this study, from the data of Hooftman *et al.* (2007) we only used the parental species, three defined generations of hybrids (F_1 , S_1 and BC_1) and one rate of hybrid vigour breakdown. Such a restriction allows a better investigation of the sensitivity of the model to single factors, restraining complex interactions between hybrid classes. We used the empirical values for the relative fitness (λ) of the respective classes: $\lambda_{serriola}$ was set to 1 (neutral population growth), and all other λ values obtained from the experiments were rescaled to values relative to *L. serriola*. One non-empirical 'hybrid swarm' class was required to include all outcrossing possibilities not resulting in one of the defined hybrid categories.

The classes included in our analysis were:

1. *L. sativa* ($\lambda = 0.27$), involved only in the formation of initial F_1 hybrids at $t = 0$; its dynamics were not modelled further.
2. *L. serriola* ($\lambda = 1$), the pure (i.e. not introgressed) wild taxon; this class reproduced either by selfing or by outcrossing with another *L. serriola* plant.

The number of individuals in every breeding class at time t (frequency) represented the number of seeds formed after (i) reciprocal crossing ($N_{outcross}$), and (ii) the number of plants in generation t from the same breeding class in generation $t - 1$ (N_{self}). The latter was the sum of the seeds produced by selfing and the seeds produced by outcrossing with a plant of the same breeding class. All breeding classes could be produced reciprocally, with i as seed producer and j as pollen donor and vice versa. For example, the BC₁ outcrossing progeny class is formed by crossing *L. serriola* with the F₁ or S-class in which all categories are used both maternal (i) and paternal (j); therefore, (I -app: equations 1 and 2) equals three classes for $k = BC_1$ (see also Table 1 and Hoofman *et al.* 2007).

To incorporate the hybrid vigour breakdown, two so-called 'loops' were constructed, representing advanced generations of hybrids. Within loops, the fitness was asymptotically reduced to a pre-set level (λ_{floor}). The S-loop represents the classes S₂ and further offspring produced by the autogamous pathway; the BC-loop represents the classes BC_{1S1} and following autogamous generations thereof. The hybrid swarm follows similar logic. The fitness within these loops decreased as follows:

$$\lambda_{i(t)after_breakdown} = \left(\left(\lambda_{i(initial)} - \lambda_{floor} \right) \times (1 - \phi)^{(t-t_b)} \right) + \lambda_{floor} \quad \text{eqn 4}$$

With $\lambda_{i(initial)}$ the initial fitness of class i at t_b , ϕ the estimated proportion of hybrid vigour breakdown generation⁻¹, λ_{floor} the assumed fitness of advanced hybrid generations within autogamous loops, t_b = the first t a loop-class can appear in (i.e. for S and HS-loops $t_b = 3$, BC_{1S}-loops $t_b = 4$).

We estimated the hybrid vigour to decrease by 26% generation⁻¹, averaging the empirically determined decrease in λ per generation along the autogamous and backcrossing pathway. In non-sensitivity calculations λ_{floor} equalled $\lambda_{L. serriola}$.

SIMULATIONS

We defined 'the number of generations to displacement' (NG-D) as the t in which the relative frequency of *L. serriola* dropped permanently below 5% of the total population. Similarly, 'the number of generations to hybrid swarm dominance' (NG-swarm) was defined as the t in which the relative frequency of the hybrid swarm exceeded 95%.

For a non-stochastic run, we calculated the relative frequencies of each of the different classes over 150 simulated generations. From the used set of parameters, the initial fitness of the classes and the hybrid vigour breakdown rate were empirically determined. All other parameters, including the fitness of advanced generation hybrids (λ_{floor}), are assumptions with no information about potential covariance among and variation within factors.

Stochasticity was introduced by changing each $\lambda(i)_{initial}$ and $T(i)$ independently per t according to equations 5 and 6. These values were subsequently transferred into equations 1 and 2.

$$\lambda_{S(i)t} = \lambda(i) + (v * \lambda(i)) \quad \text{eqn 5}$$

$$T_{S(i)t} = T + (v * T) \quad \text{eqn 6}$$

With $\lambda_{S(i)t}$ stochastic fitness at t for class i ; $\lambda(i)$ empirically defined fitness for class i ; $T_{S(i)t}$ stochastic outcrossing rate at t for class i ; T = outcrossing estimate. v is a random value following a normal distribution with average 0 and as standard deviation the level of stochasticity (%).

SENSITIVITY ANALYSES

Dependence of NG-D and NG-swarm on different model parameters (non-stochastic)

NG-D and NG-swarm are related to the outcrossing rate through performing simulations at T -intervals of 0.01%. Similarly, NG-D and T are correlated to four different factors assumed to be of high importance, depicting their joint correlation per pair to NG-D as an interaction surface. Factors tested were (i) the hybrid vigour breakdown rate (ϕ), (ii) the fitness asymptote for advanced generation hybrids (λ_{floor}), (iii) the starting ratio of crop to wild plants, and (iv) the temporal frequency of crop occurrence (e.g. once every 10 years).

Altering single class transitions at different levels of stochasticity. Since our model was non-linear, direct matrix elasticity analysis could not be performed analogous to, for example, Garnier & Lecomte (2006) and Sebert-Cuvillier *et al.* (2007). Therefore, we defined sensitivity as follows: 'the average proportional change, caused by changing a single parameter or transition, of the growth rate of the simulated population as a whole ($\lambda_{population}$) or subgroups within it ($\lambda_{L. serriola}$; $\lambda_{all hybrids}$)'. The sensitivities of among-class transitions were expressed in relative terms that sum to 1, and are therefore comparable to regular elasticities. Still we will refer to our measure as sensitivity instead of elasticity, since the latter refers to a specific equation in matrix modelling.

Sensitivity of the growth of the whole population or of particular subgroups (e.g. $\lambda_{L. serriola}$ or $\lambda_{all hybrids}$) to changes in a class transition (P_c) was calculated according to equations 7 and 8 using two subsequent stochastic simulations of 1000 runs each. To include sensitivity, ζ was used (equations 1 and 2). In the first stochastic simulation, the transition rates are unchanged ($\zeta = 0$). All $\lambda_{S(i)t}$ and $T_{S(i)t}$ of this run were stored to enable the performance of an identical set of runs after changing a transition or parameter value in a second simulation (Tuljapurkar, Horvitz & Pascarella 2003). In the second simulation, these previously generated $\lambda_{S(i)t}$ and $T_{S(i)t}$ values were again used but with the respective transition raised by 25% ($\zeta = 0.25$). More specifically, this was done for those particular combinations of i and j that produced progeny of class k . For all other combinations, ζ was kept at 0. This was done for one transition at the time. We constrained among-class transitions to values between 0–1.

Stochastic sensitivity (P_c) =

$$\left[\frac{\left(\frac{\sum_{t=1}^{t_{max}} \left(\frac{1}{t_{max}} \times \sum_{t=1}^{t_{max}} (\chi^{ps} - 1) \right)}{r_{max} \times \zeta} \right)}{\sum_{p=1}^{p_{max}} \left(\frac{\sum_{t=1}^{t_{max}} \left(\frac{1}{t_{max}} \times \sum_{t=1}^{t_{max}} (\chi^{ps} - 1) \right)}{r_{max} \times \zeta} \right)} \right] \quad \text{eqn 7}$$

$$\chi^{ps} = \begin{cases} \chi^p & \text{if } \chi^p \geq 1 \\ \frac{1}{\chi^p} & \text{if } \chi^p < 1 \end{cases} \quad \text{eqn 8}$$

in which $\chi^p = \frac{\left(N(c)_t^p \times \frac{1}{N(c)_{t-1}^p} \right)}{\left(N(c)_t \times \frac{1}{N(c)_{t-1}} \right)}$

With P the among-class transition or parameter for which sensitivity is tested (P_{max} : all parameters tested in one analysis); c a (sub-) population of individuals (i.e. whole population, *L. serriola* or hybrids) affected by changing P ; $N(c)_t$ the number of individuals at t of c in stochastic simulations without alterations in parameters; $N(c)_t^P$ the number of individuals at t of c in stochastic simulation in which P has a raised value by ζ ; χ^P the proportional difference (≥ 1) in population growth rate of c caused by changing P ; t_{max} the number of generations tested (= 150); r a single model run and r_{max} the number of runs performed (= 1000). ($N(c)_t/N(c)_{t-1}$) could be considered the growth rate of c (λ_c) at t .

Altering single parameters at different levels of stochasticity.

Among-class transitions are complexes of parameters; therefore, changes in parameters occurring in multiple among-class transitions could either cancel out or reinforce each other. For that reason, a further analysis assessed the sensitivity of the growth rates of *L. serriola* or all hybrid classes ($\lambda_{L. serriola}$ or $\lambda_{all\ hybrids}$) to changes in the following eight parameters: T , ϕ , λ_{floor} and the initial fitness values ($\lambda_{(initial)}$) for all classes before hybrid vigour breakdown. This analysis followed the above method in which not a transition but the parameter is raised with a factor ζ in the second stochastic simulation. Decreasing parameter values instead of increasing gave similar patterns and therefore this analysis is not presented here.

Results

NON STOCHASTIC SIMULATION

In a non-stochastic simulation (Fig. 1), *L. serriola* is displaced by hybrids to a frequency < 5% after 61 years (< 0.1% after 139 years). The hybrid swarm reached dominance (> 95%) after 80 generations, suppressing other hybrid classes. Since the fitness of advanced hybrids equalled that of *L. serriola*, the replacement of the wild taxon might be unexpected. Its main cause is ‘drainage’ into the hybrid swarm: many parental combinations actually result in offspring that end up in the hybrid swarm.

DEPENDENCE OF NG-D AND NG-SWARM ON DIFFERENT MODEL PARAMETERS

NG-D and NG-swarm depended on the outcrossing rate (Fig. 2). A prolonged coexistence of *L. serriola* and hybrids (i.e. for more than 150 generations) was possible at low outcrossing rates ($T \leq 2.5\%$). At higher outcrossing rates,

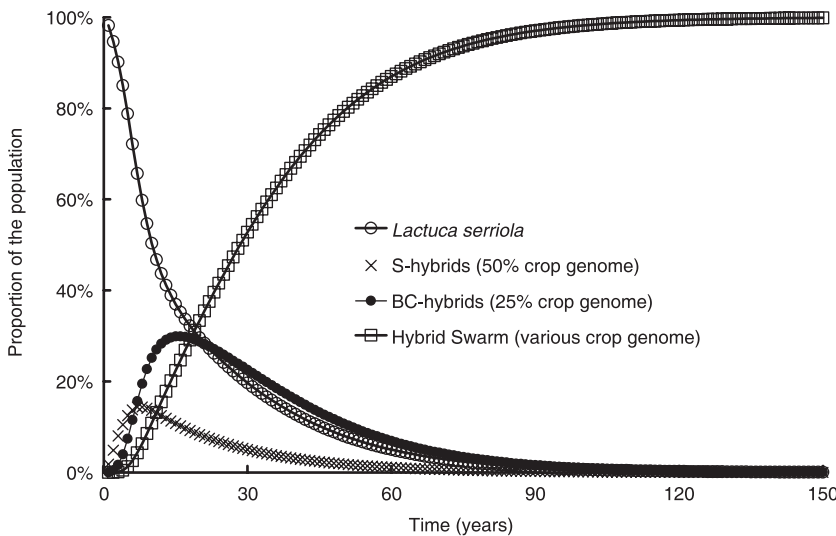


Fig 1. Non-stochastic model simulation after a single hybridization event at $t=0$ with a pollen flow of *L. sativa* from an equal population size as of *L. serriola*. An outcrossing rate of 5% was assumed. Classes shown are based on the average containing proportion of crop genome; the hybrid swarm includes all hybrids, which can not be genomically assigned to one of the other classes. Hybrid classes are subject to a 26% fitness reduction generation⁻¹ towards λ_{floor} ; λ_{floor} equals $\lambda_{L. serriola}$ (= 1).

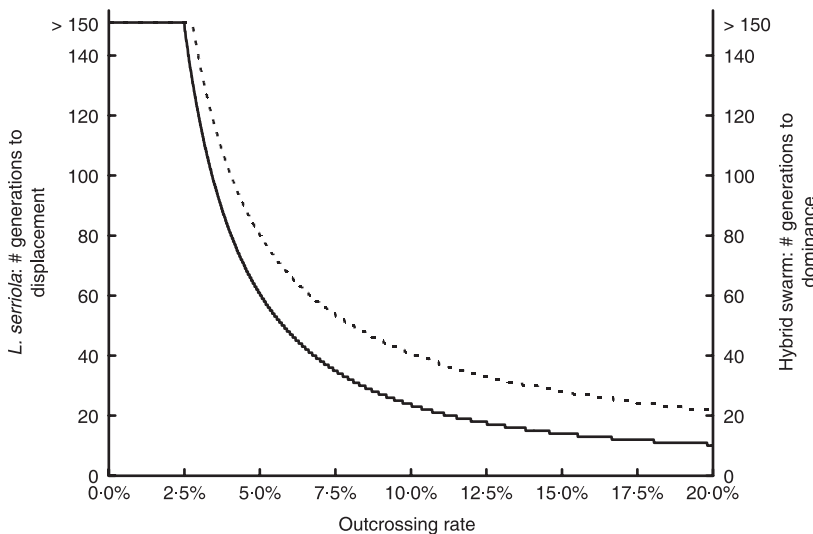


Fig. 2. Displacement of *L. serriola* (NG-D) and dominance of the hybrid swarm (NG-swarm, dotted line) (defined as relative frequencies of < 5% and > 95%, respectively) as function of outcrossing rate. Step size is 0.01%.

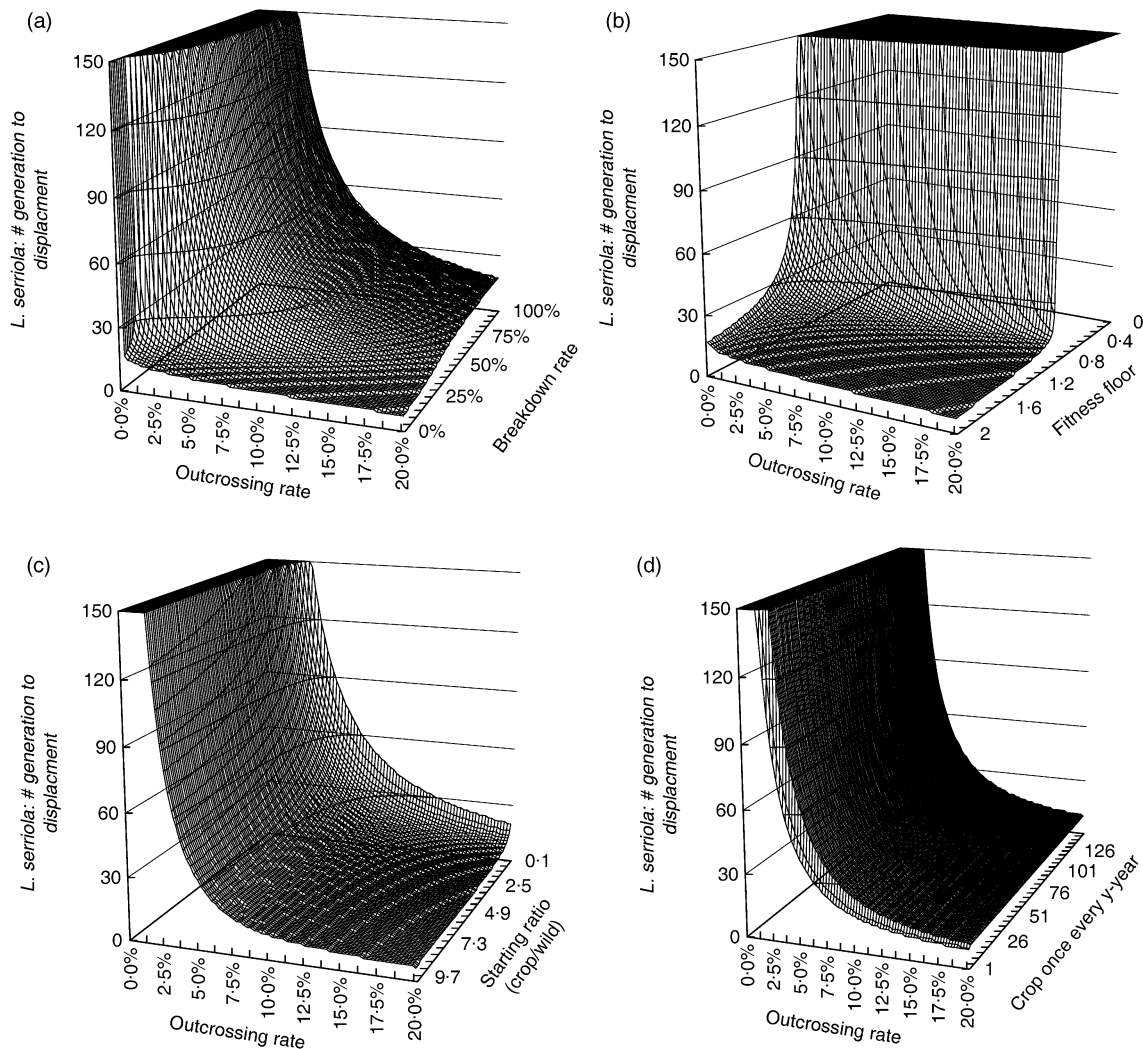


Fig. 3. Interaction surfaces for the estimated number of generations to displacement (NG-D) of *L. serriola* as a function of outcrossing rate and (a) hybrid vigor breakdown rate generation⁻¹ (ϕ); (b) the assumed fitness of advanced hybrid generations within autogamous loops (λ_{floor}); (c) starting ratio in no. of plants at $t = 1$ (*L. sativa*/*L. serriola*); (d) frequency of *L. sativa* presence. NG-Ds ≥ 150 generations are considered as no displacement. The shown grid represents the employed parameter step size.

displacement of *L. serriola* occurred well before 150 generations (NG-D = 10 at $T = 20\%$). Both trajectories had a rather steep slope at lower outcrossing rates ($T < 10\%$) and flattened at higher outcrossing rates being asymptotic to three and six generations for NG-D and NG-swarm, respectively (Fig. not shown). The delay in hybrid swarm dominance compared to *L. serriola* displacement was caused by the presence of other hybrid classes.

A prolonged *L. serriola* existence required a hybrid vigour breakdown-rate above approximately 8% (Fig. 3a), indicating the importance of adding a level of vigour breakdown into models. Note that this rate is considerably lower than the empirically defined breakdown rate of 26% in our lettuce hybrids. Furthermore, an increase of NG-D with increasing breakdown rate along T -clines suggested an interaction between both parameters in which a higher breakdown rate buffers NG-D, to some extent, at higher outcrossing rates.

Alterations in the fitness asymptote to which the hybrid vigour eventually decreased in advanced hybrid generations

(λ_{floor}) highly affected NG-D (Fig. 3b). Prolonged coexistence of *L. serriola* and hybrids was probable only for λ_{floor} values between 0.82 and 1.04 (i.e. if the fitness of advanced generation hybrids was between 82–104% of the fitness of *L. serriola*). Within this range of λ_{floor} , NG-D depended only to a low extent on the outcrossing rate. Above 1.04, *L. serriola* was displaced within 150 years, independent of the outcrossing rate. On the contrary, when $\lambda_{\text{floor}} \leq 0.8$ hybrids (after their initial formation) were gradually displaced by the wild taxon (Fig. not shown).

In contrast to the breakdown rate and the fitness asymptote, the species starting ratio (*L. sativa*/*L. serriola*; Fig. 3c) and the frequency of crop presence in time (Fig. 3d) had little effect on *L. serriola* displacement, irrespective of the outcrossing rate.

SENSITIVITIES TO TRANSITION RATES AT DIFFERENT LEVELS OF STOCHASTICITY

The growth rate λ of the whole population ($\lambda_{\text{population}}$) was most sensitive to changes in the fitness of the autogamous loops

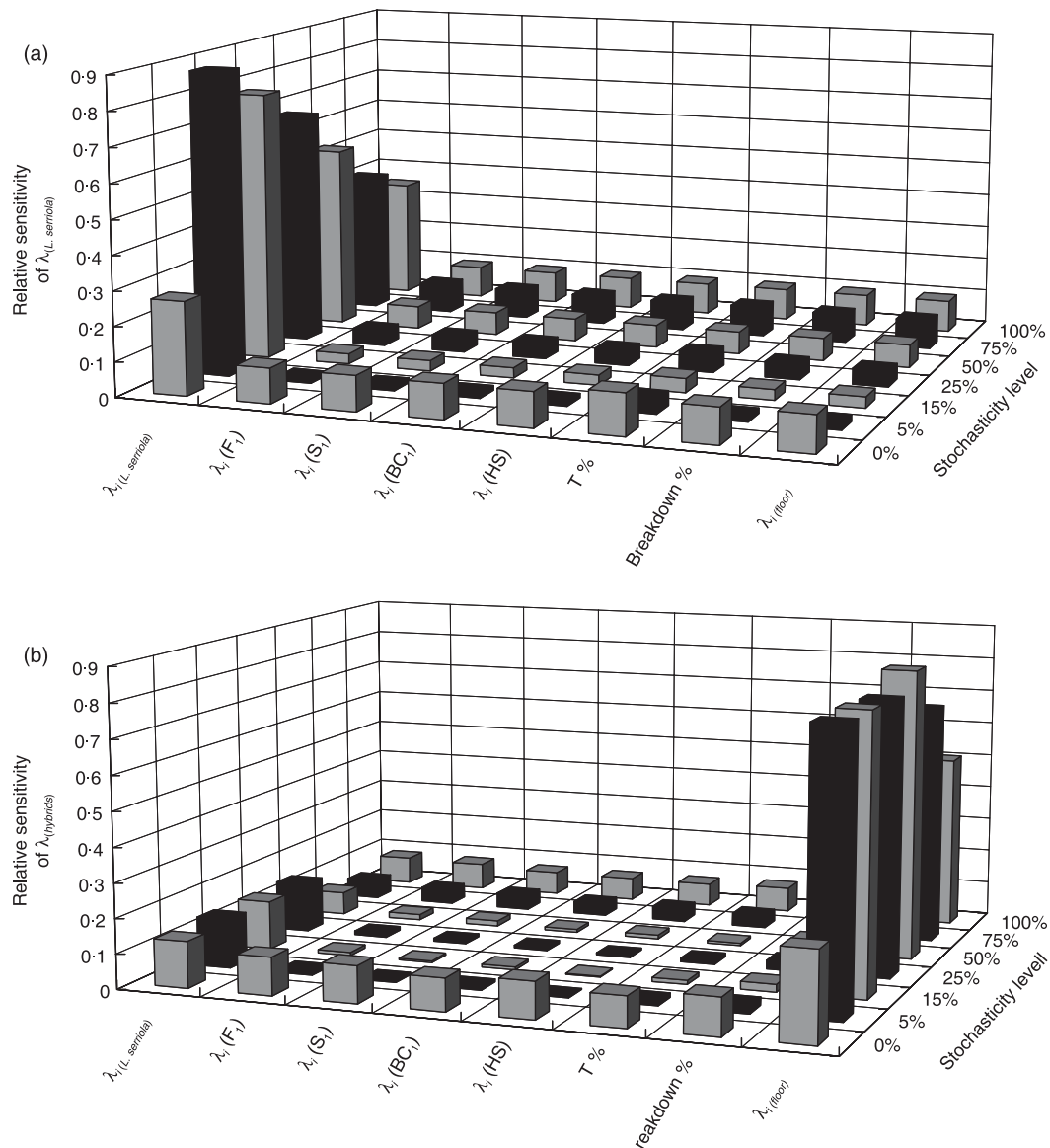


Fig. 4. The effect of the stochasticity rate on the relative sensitivity (sum = 1 per stochasticity level) on the growth rate of *L. serriola* ($\lambda_{L. serriola}$) (a) and the growth rate of the hybrid classes ($\lambda_{all hybrids}$); (b) to changes in the initial class fitness (λ_i); the estimated outcrossing rate (5%), the hybrid breakdown rate generation⁻¹ (ϕ) and the assumed fitness of advanced hybrid generations within autogamous loops (λ_{floor}).

(*L. serriola*, S-loop, BC₁-loop and hybrid swarm) and comparatively insensitive to changes in the transition rates that produced the distinct hybrid classes F₁, S₁ and BC₁ via crossing events (Table 1).

The inclusion of stochasticity had little effect on the sensitivity of population growth to changes in class transitions, with the exception that changes in transitions that produced *L. serriola* progeny had a lower impact on population growth if stochasticity was high (Table 1).

SENSITIVITIES TO INDIVIDUAL PARAMETERS AT DIFFERENT LEVELS OF STOCHASTICITY

The growth rate of *L. serriola* ($\lambda_{L. serriola}$) and that of all hybrids ($\lambda_{all hybrids}$) in the population were most sensitive to changes in the initial fitness of *L. serriola* ($\lambda_{i(L. serriola)}$); Fig. 4a) and in the

fitness asymptote for advanced generation hybrids (λ_{floor} ; Fig. 4b), respectively. All other parameters contributed only little to growth rate changes. At low to moderate stochasticity levels, the disproportionate impact of changes in $\lambda_{i(L. serriola)}$ and λ_{floor} (Fig. 4) was strongly enhanced. At higher stochasticity levels, the contribution of all parameters to the sensitivity of the growth rates became more even. This effect was stronger for $\lambda_{L. serriola}$ and was similar to the effect found for the *L. serriola* loop transition (Table 1). These results, in combination with Fig 3a, suggest that the composition of the population is mainly determined by an interaction between the fitness of *L. serriola* and that of advanced hybrids (λ_{floor}). Other parameters, including T , had a lower proportional effect on the average population growth rates of hybrids and *L. serriola*, although larger scale changes could still have major effects (Fig. 2).

Discussion

Risk assessments of the potential environmental impact of new (transgenic) crops need to be supplied with the most relevant data (Snow *et al.* 2005). The goal of our modelling work is to guide data collection towards factors which might have the largest impact on the long-term consequences of hybridization between crops and wild relatives.

In our simulations, we found that displacement of *L. serriola* strongly depends on assumptions about the fitness of advanced-generation hybrids relative to the fitness of the wild taxon. In cases in which both the fitness of early and advanced generation hybrids was equal or higher than *L. serriola*, displacement of the wild taxon was highly likely, and largely independent of the outcrossing rate.

Coexistence of *L. serriola* and hybrids was demonstrated to be probable if the outcrossing rate was low and the hybrid vigour unstable over time (Hooftman *et al.* 2007, but see Campbell, Galen & Wu 2005; Erickson & Fenster 2006). Although the outcrossing rate affected the estimated population composition strongly, the proportional impact of changes in the outcrossing rate was low compared to the effect of changes in the fitness of *L. serriola* and of advanced generation hybrids. This was also reflected in the insensitivity of the population growth rate to changes in the transition rates involving among-genotype crossing. Furthermore, changes in the fitness of early generation hybrids, the starting ratio of the classes, and in the temporal frequency of crop occurrence had minor effects on the population growth rate. Nevertheless, it can be expected that continuous exchange (i.e. not only a single outcrossing event) would lead to a more genotypically diverse population.

STOCHASTICITY

Ruderal species such as *L. serriola* live in dynamic and highly stochastic habitats, depending on continuous anthropogenic disturbance (Freckleton & Watkinson 2002a). Hence, it is important to include stochasticity into models on wild species displacement and hybrid occurrence (Claessen *et al.* 2005). Addition of stochasticity prolonged the co-occurrence of hybrids and *L. serriola*, depending on the assumed stochasticity level (Hooftman *et al.* 2007). Hooftman and co-workers identified an equilibrium situation in which the occurrence of both hybrids and *L. serriola* was predicted to be likely at high levels of stochasticity for most tested outcrossing rates ($\leq 20\%$), balancing the numbers of plants through few good years. Here we see that this effect was due to the (sub-) population dynamics of *L. serriola* becoming less sensitive to changes in fitness at high stochasticity levels. Still, stochasticity had only relatively little effect on most other parameters.

THE OUTCROSSING RATE

Outcrossing probabilities have been considered a prominent feature in risk assessment (Ellstrand 2003), especially when high. The opposite (i.e. low outcrossing rates) could con-

sequently be interpreted as a characteristic of 'safe' species. This focus seems only partly supported by the results of our studies. Large alterations in outcrossing rate caused substantial effects on the population dynamics but, proportionally, other factors seem more important such as the fitness of advanced hybrid generations. Consequently, the autogamous (i.e. self-fertilizing) pathways seem much more sensitive than the (back)crossing pathways. Moreover, since all autogamous species have some degree of outcrossing, even after a rare outcrossing event a single F_1 individual may produce numerous autogamous hybrid progeny (Snow 2002; Song *et al.* 2003). Therefore, low outcrossing rates will enable co-occurrence of hybrids and wild relatives for longer periods of time but will not act against gene introgression as such. This conclusion holds for *Lactuca*, but may hold equally well for worldwide important and predominantly autogamous crops like *Oryza* and *Triticum*.

CLASS-BASED MODELS AND INDIVIDUAL FITNESS

In matrix-based models like ours, plants are lumped into broad classes of hybrids, classes for which it is feasible to obtain empirical data for a large number of plants (Bullock 1999; Gray 2004; Hooftman *et al.* 2005). Class-based fitness estimates should not be confused with individual plant fitness, however (Hall, Hastings & Ayres 2006). Since each class is represented by an average value, the inheritance and impact of individual genes cannot be estimated. Segregation and recombination in (partly) heterozygous individuals will constantly result in new variants, including phenotypes that might deviate substantially from the average (Rieseberg, Archer & Wayne 1999; Burke & Arnold 2001). Different modelling techniques, such as Individual Based Modelling (Grimm & Railsback 2005) would be necessary for such more advanced analyses. However, such models need substantially more data at the individual plant level.

METAPOPULATIONS AND THE BIOTIC ENVIRONMENT

Spatial factors did not receive much attention until recently (e.g. Claessen, Gilligan & van den Bosch 2005; Garnier, Deville & Lecomte 2006) and could be developed further as an important line of future research. The spread of introgressants could be a combination of local take-over of populations and dispersal to new, neighbouring populations, depending on the structure of the landscape (Freckleton & Watkinson 2002a). For *L. serriola*, single populations might be unstable and transient, since this taxon tends to form patchy metapopulations in which only part of the patches (= potentially suitable sites) is occupied each year (Freckleton & Watkinson 2002b).

Demographic processes might also depend on interactions between each breeding class and the biotic environment (Keller, Kollmann & Edwards 2001; Johnston *et al.* 2001; Pilon & Prendeville 2004). In *Brassica*, for instance, lowered competitive ability of introgressants was found in dense populations (Hauser, Damgaard & Jørgensen 2003; Johannessen

et al. 2006). When data are available, it seems feasible to fit such relationships into models like Thompson *et al.* (2003), Hall, Hastings & Ayres (2006) or Hooftman *et al.* (2007).

IMPLICATIONS

Combining our conclusions for outcrossing rate and hybrid vigour breakdown, we make an important addition to the theoretical predictions that displacement is mostly dependent on (hybrid) offspring fitness, as suggested by Huxel (1999) and Haygood, Ives & Andow (2003). We find that the offspring fitness of *advanced generations* affects the consequences of hybridization most, not the fitness of *early hybrid generations*, as usually thought (Johansen-Morris & Latta 2006).

The potential impact of few genes (or combination of genes) providing a stable increase in fitness could be important for their spread and persistence in recipient populations beyond the effects of hybridization (Rieseberg & Burke 2001; Hails & Morley 2005; Guadagnuolo, Clegg & Ellstrand 2006). Also, it seems ineffective to consider traits that lower *individual* plant fitness, such as terminator constructions or dwarfing genes (e.g. Al-Ahmad *et al.* 2006), as a mitigation measure to decrease *population* fitness. A progeny line, which does not express a suppressed phenotype because of mutation or recombination, will quickly displace all other hybrid lineages (Hall, Hastings & Ayres 2006), making potential displacement of the wild relative independent of the mitigation construct.

From an applied perspective, we suggest that future risk assessments should focus much more on long-term hybrid fitness instead of early generation fitness, by conducting experiments that include later generations. Decision-making frameworks would benefit from information about the long-term effects of the introduction of transgenes (Snow *et al.* 2005; Andow & Zwahlen 2006). Annuals like *Lactuca*, producing at least two generations per year in the greenhouse, would be the most tractable model systems. Long-term generation studies could provide better estimations of hybrid vigour breakdown rates and advanced hybrid fitness. Furthermore, long-term experiments would provide information on which traits are likely to introgress, thereby enabling potentially harmful hybrids to be identified in the field after crop release.

Acknowledgements

We like to thank Peter van Tienderen, Jacqueline Kummer, our partners in ANGEL and all participants of the workshops in Dorset (UK), Nøbedo (Denmark) and Sogndal (Norway) for all discussions. Christian Damgaard, Rosie Hails, Eric Schranz and two anonymous reviewers are acknowledged for constructive comments on an earlier version of this manuscript. This study was supported by EC-grant QLK3-2001-01657 to J.N.

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Received 10 January 2008; accepted 14 May 2008

Handling Editor: Rosie Hails