

How molecular tools can help understanding species interactions to improve restoration outcomes

Stéphane Boyer^{1,2,*}, Benjamin R. Waterhouse², and Steve D. Wratten²

¹Environmental and Animal Sciences, Unitec Institute of Technology, 139 Carrington Road, Mt Albert, Auckland 1025, New Zealand

²Bio-Protection Research Centre, Lincoln University, P.O. Box 85084, Lincoln 7647, New Zealand

*stephane.boyer@gmail.com

ABSTRACT

Preserving species interactions should be a key desired outcome in restoration ecology. With progress in environmental DNA techniques and the dramatic reduction in the cost of high-throughput DNA sequencing, large amounts of information can be gathered on how species interact with little to no disturbance to ecosystems. Here, we argue that the use of molecular tools to study ecological interactions will become increasingly important in restoration projects. We describe specific examples where recent advances in genetics allow for a better understanding of predator-prey, animal-plant, plant-microbe and trophic cascade interactions, which can inform restoration practice and substantially improve our capacity to restore functioning ecosystems.

Key words: food webs, trophic cascades, genetics, high-throughput DNA sequencing.

Highlights

- Maintaining species interactions should be a key desired outcome in restoration ecology.
- Molecular tools provide means to measure, quantify and monitor species interactions at minimum costs and with little to no interference to the monitored ecosystem.
- In particular, recent advances in genetics allow for a better understanding of predator-prey, animal-plant, plant-microbe and trophic cascade interactions.
- The use of molecular tools remains scarce in ecological restoration studies.
- There is a need for environmental legislation to adopt requirements around the use of genetic information to design restoration programmes and measure restoration success.

Introduction

Ecological restoration programmes and the evaluation of restoration success are largely based on the abundance and diversity of species in the restored areas compared to a historic or reference state [1–3]. Because ecosystems are not defined only by the physical environment and the species they contain but also by their interactions, maintaining such interactions should also be a key desired outcome in restoration ecology. One of the 11 attributes for successful restoration proposed by The Society for Ecological Restoration (SER 2004) is the normal functioning of the restored ecosystem, which in many cases can be achieved only through the restoration of ecological interactions. The latter can be complex and difficult to quantify in natural ecosystems. However, recent progress in environmental DNA techniques [5] and the accessibility of high-throughput DNA sequencing, provide means to measure, quantify and monitor species interactions at minimum costs and with little to no interference to the monitored ecosystem. Despite this, the use of molecular tools remains scarce in ecological restoration studies as demonstrated by the low proportion of genetic studies published in the journal *Restoration Ecology*: 5% in 2015 (Fig. 1A). Similarly, only 7% of the oral presentations at the latest edition of the main international conference in ecological restoration (SER2015) had any reference to genetic or molecular tools (Fig. 1B). A recent article by Mijangos et al. (2015) reviewed applied ecological studies, which exemplified the broad range of restoration interventions and objectives in which molecular tools have been used. The majority of the reviewed studies focused on intraspecific genetic diversity and the associations between genetics

and fitness, while a smaller proportion assessed demographic issues and very few investigated the link between genetics and the restoration of ecosystem services. The latter refers to studies where increased genetic diversity led to better restoration success, as measured by enhanced ecosystem services [7,8]. Restoration studies so far have seldom used genetic approaches to understand how species interact and there is a need to emphasise how novel molecular approaches can increasingly be used to better understand species interactions and inform restoration plans [9] or assess restoration success. Here we outline four main types of interspecific interactions for which molecular approaches can better inform ecological restoration projects (Fig. 2).

Predator-prey interactions

In the past decade, genetic tools have greatly increased our capacity to investigate predator-prey interactions [10,11]. These tools can also be applied to inform restoration programmes and ensure restoration of functioning trophic interactions. For example, as part of a mine restoration project, Boyer et al. (2013) used a next-generation sequencing approach to quantify the diet of an endangered carnivorous landsnail feeding on endemic earthworms. The results were used to set appropriate goals for the restoration of a diverse earthworm community. In particular, the molecular analysis highlighted the importance of restoring earthworm communities comprising leaf litter (epigeic) and deep burrowing (anecic) species in the snails' original habitat. A similar study on a different landsnail species highlighted the need to consider seasonal changes in diet when restoring the habitat of an endangered species [13]. Technologies used for molecular detection of trophic interactions, and the ecological questions that can now be addressed using these tools, are growing exponentially [5]. This rapidly developing field will generate valuable information about ecological restoration processes. For example, by combining genetic tools and isotopic analyses, it is possible to draw very accurate 'maps' of which species recolonize early-recovering ecosystems, where they originate and how they share the resources and interact at different stages of ecological succession [14].

Animal-plant interactions

Genetics can also be used to elucidate interactions between plants and animals, some of which have direct impact on restoration outcomes. For example, bird contribution to natural regeneration through seed rain can be significant in restoration projects [15], particularly during the critical first decade of recovery, when lack of seed dispersal may be limiting vegetation regeneration [16]. However, the contribution to seed dispersal varies greatly from one bird species to another (e.g.17). Molecular analyses of bird faeces in which freshly dispersed seeds are embedded have been used to identify which bird species contributes to the dispersal of which plant species [18]. Using this method, it will become much easier to quantify the contribution of various seed-dispersing species and better inform decisions regarding species re-introduction for improved ecological functions or ecological replacement programmes. In a similar way, pollination by honeybees can be estimated by using molecular tools to analyse the pollen pellets they collect and identify the plant species they interact with [19]. This has the potential to greatly simplify the assessment of pollinator contributions to restoring flowering plant communities. The application of high-throughput DNA sequencing methods to analyse pollen collected by bees will further elucidate these relationships. These tools can be used to identify plant species that seldom benefit from bee pollination and may require targeted restoration measures. In addition, pollination by other animals, which may be difficult to observe in the wild (such as nocturnal pollinators) could be accurately diagnosed using molecular methods [20]. Knowledge gained through molecular analyses of such interactions will help better to predict plant recruitment and recolonisation rates through seed deposition and pollination patterns in relation to the seed-dispersing animals and pollinators present in the environment.

Plant-microbe interactions

Microbial communities including nitrogen-fixing bacteria, plant-growth promoting rhizobacteria, mycorrhizal helper bacteria, and arbuscular mycorrhizal fungi (AMF) also have the potential to greatly accelerate restoration through their interactions with plants. Because of their key impact on plant survival and growth, these organisms have been well studied in the restoration literature [21–24]. AMF for example, which form associations with the roots of 80% of vascular plant species [25], can improve plant growth [26,27], resistance to pathogens [28] and invertebrate herbivores [29], phytoremediation activity [30] and establishment of native vegetation [21]. The application of genetic tools has shown that plant and AMF communities are tightly coupled in their response to ecosystem development [31]. However, AMF can also have detrimental effects on plants [32,33]. The integration of the well-developed microbial 'OMICS' tools to better understand and differentiate beneficial/detrimental interactions will undoubtedly be a major area of restoration ecology research in the coming years [34].

Trophic cascades and complex interaction networks

While the aim should be to preserve functional ecosystems (SER 2004), current restoration practice often fails to recover original rates of ecosystem functions [35]. Many ecosystem functions are directly influenced by ecological interactions and

assessment of these before and after restoration is essential to set up restoration goals and evaluate success. These interactions include pollination, seed dispersal, nitrogen fixation and other microbial symbioses as developed in the examples above, but also top-down control of keystone species or ecological engineers by direct predation or through more complex trophic cascades. For example, selective grazing of microbial species by soil invertebrates is determinant in the structuring of fungal and bacterial communities [36] and microbe–grazer interactions can moderate soil decomposer community function [37], thereby influencing plant growth. A deep understanding of the interactions between arthropod grazers and fungal communities will lead to better predictions of decomposition rates, nutrient cycling and eventually plant growth rates in restored areas. Molecular tools capable of disentangling such complex interactions networks are on the horizon. For example, tri-trophic interactions have been described in the form of secondary predation in an aphid–spider–carabid interaction where carabid beetles were tested positive for aphid DNA for up to 8 h after predating spiders that had fed on aphids [38]. Although secondary predation was originally considered as a potential issue in diet analyses, increasing sensitivity of molecular methods and the capacity to detect minute amounts of DNA opens considerable opportunities in food-web ecology. For example, Paula et al. (2015) analysed gut samples from harlequin ladybirds, and sequenced DNA from the predator (ladybird) and its prey (aphid) as well as that of bacterial symbionts of the prey. In another study, Varennes et al. (2014) developed a molecular method that has the potential to detect host, parasitoid and hyperparasitoid DNA from aphid mummies and identify the different species involved in this tri-trophic interaction. Although the above studies were conducted under laboratory conditions, increasing sensitivity of molecular methods and better understanding of DNA decay rates across multiple trophic levels (e.g. Paula et al. 2015) will probably shed light on complex relationships and the dynamics of interactions in natural ecosystems.

Conclusion

Although restoring community composition and maintaining species genetic diversity are obvious objectives in ecological restoration programmes, as illustrated in the recent review by Mijangos et al. (2015), the restoration of conditions that allow species interactions to be maintained or restored is also essential as it may often be determinant to restoration outcomes [41]. Multitrophic functional diversity is likely to be a better indicator of ecosystem functioning than the commonly used species richness [42], and understanding multi-trophic and site-specific food webs at the onset of degradation will be an essential component of future restoration projects [1]. To ensure that restored ecosystems function provide desired ecosystem services, the focus needs to be on conserving whole communities and their interactions rather than a restrictive set of charismatic species [43]. Continuous development in molecular ecology will greatly contribute to achieve this goal. Modern high-throughput DNA sequencing methodologies combined with a broader use of environmental DNA and construction of robust and ever-increasing sequence databases are sure to contribute to more accurate restoration goals and better prediction of restoration outcomes. However, to achieve direct impacts on restoration success, there is a need for environmental legislation to adopt requirements around the use of genetic information to design restoration programmes and measure restoration success.

References

1. Fraser, L. H. et al. A call for applying trophic structure in ecological restoration. *Restor. Ecol.* 23, 503–507 (2015).
2. Ruiz-jaen, M. C. and Aide, T. M. Restoration success: how is it being measured? *Restor. Ecol.* 13, 569–577 (2005).
3. Wortley, L., Hero, J. M. and Howes, M. Evaluating ecological restoration success: A review of the literature. *Restor. Ecol.* 21, 537–543 (2013).
4. Society for Ecological Restoration International Science and Policy Working Group. The SER International primer on ecological restoration. 2, (2004).
5. Symondson, W. O. C. and Harwood, J. D. Special issue on molecular detection of trophic interactions: unpicking the tangled bank. Introduction. *Mol. Ecol.* 23, 3601–4 (2014).
6. Mijangos, J. L., Pacioni, C., Spencer, P. B. S. and Craig, M. D. Contribution of genetics to ecological restoration. *Mol. Ecol.* 24, 22–37 (2015).
7. Reynolds, L. K., McGlathery, K. J. and Waycott, M. Genetic diversity enhances restoration success by augmenting ecosystem services. *PLoS One* 7, e38397 (2012).
8. Ritchie, A. L. and Krauss, S. L. A Genetic Assessment of Ecological Restoration Success in *Banksia attenuata*. *Restor. Ecol.* 20, 441–449 (2012).

9. Clare, E. L. Molecular detection of trophic interactions, emerging trends, distinct advantages, significant considerations and conservation applications. *Evol. Appl.* 7, 1144–1157 (2014).
10. Symondson, W. O. C. Molecular identification of prey in predator diets. *Mol. Ecol.* 11, 627–641 (2002).
11. King, R. A., Read, D. S., Traugott, M. and Symondson, W. O. C. Molecular analysis of predation: a review of best practice for DNA-based approaches. *Mol. Ecol.* 17, 947–963 (2008).
12. Boyer, S., Wratten, S. D., Holyoake, A., Abdelkrim, J. and Cruickshank, R. H. Using Next-Generation Sequencing to Analyse the Diet of a Highly Endangered Land Snail (*Powelliphanta augusta*) Feeding on Endemic Earthworms. *PLoS One* 8, e75962 (2013).
13. Waterhouse, B. R., Boyer, S. and Wratten, S. D. Pyrosequencing of prey DNA in faeces of carnivorous land snails to facilitate ecological restoration and relocation programmes. *Oecologia* 175, 737–46 (2014).
14. Raso, L. et al. Intraguild predation in pioneer predator communities of alpine glacier forelands. *Mol. Ecol.* 23, 3744–54 (2014).
15. Zhang, H. and Chu, L. M. Seed rain and seedling survival are major factors limiting vegetation regeneration on rehabilitated quarries. *Landsc. Ecol. Eng.* 29–38 (2013). doi:10.1007/s11355-013-0231-x
16. Reid, J. L., Holl, K. D. and Zahawi, R. a. Seed dispersal limitations shift over time in tropical forest restoration. *Ecol. Appl.* 25, 1072–1082 (2015).
17. Wotton, D. M. and Mcalpine, K. G. Seed dispersal of fleshy-fruited environmental weeds in New Zealand. *N. Z. J. Ecol.* 39, In Press (2015).
18. González-Varo, J. P., Arroyo, J. M. and Jordano, P. Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods Ecol. Evol.* 5, 806–814 (2014).
19. Galimberti, A. et al. A DNA barcoding approach to characterize pollen collected by honeybees. *PLoS One* 9, e109363 (2014).
20. Clare, E. L., Schiestl, F. P., Leitch, A. R. and Chittka, L. The promise of genomics in the study of plant-pollinator interactions. *Genome Biol.* 14, 207 (2013).
21. Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P. and Barea, J. M. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Appl. Environ. Microbiol.* 67, 495–8 (2001).
22. Ma, Y., Dickinson, N. M. and Wong, M. H. Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. *Soil Biol. Biochem.* 38, 1403–1412 (2006).
23. Hong, S. H. and Lee, E. Y. Vegetation restoration and prevention of coastal sand dunes erosion using ion exchange resins and the plant growth-promoting rhizobacteria *Bacillus* sp. SH1RP8 isolated from indigenous plants. *Int. Biodeterior. Biodegradation* 95, 262–269 (2014).
24. de-Bashan, L. E., Hernandez, J. P. and Bashan, Y. The potential contribution of plant growth-promoting bacteria to reduce environmental degradation - A comprehensive evaluation. *Appl. Soil Ecol.* 61, 171–189 (2012).
25. Landeweert, R., Hoffland, E., Finlay, R. D., Kuyper, T. W. and van Breemen, N. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends Ecol. Evol.* 16, 248–254 (2001).
26. Porrás-Soriano, A., Soriano-Martín, M. L., Porrás-Piedra, A. and Azcón, R. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.* 166, 1350–1359 (2009).
27. Moora, M., Opik, M., Sen, R. and Zobel, M. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. *Funct. Ecol.* 18, 554–562 (2004).
28. Liu, J. et al. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *Plant J.* 50, 529–44 (2007).

29. Kempel, A., Schmidt, A. K., Brandl, R. and Schädler, M. Support from the underground: Induced plant resistance depends on arbuscular mycorrhizal fungi. *Funct. Ecol.* 24, 293–300 (2010).
30. Khan, A. G. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. trace Elem. Med. Biol.* 18, 355–64 (2005).
31. Martínez-García, L. B., Richardson, S. J., Tylaniakis, J. M., Peltzer, D. A. and Dickie, I. A. Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. *New Phytol.* 205, 1565–1576 (2015).
32. Johnson, N. C., Graham, J. H. and Smith, F. a. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol.* 135, 575–585 (1997).
33. Williams, A., Norton, D. and Ridgway, H. J. Different arbuscular mycorrhizal inoculants affect the growth and survival of *Podocarpus cunninghamii* restoration plantings in the Mackenzie Basin, New Zealand. *New Zeal. J. Bot.* 50, 473–479 (2012).
34. Waterhouse, B. R. Conserving New Zealand’s endemic carnivorous land snail *Powelliphanta patrickensis* through restoration of a functioning by. (2014).
35. Moreno-Mateos, D., Power, M. E., Comín, F. a. and Yockteng, R. Structural and functional loss in restored wetland ecosystems. *PLoS Biol.* 10, (2012).
36. A’Bear, A. D., Jones, T. H. and Boddy, L. Size matters, What have we learnt from microcosm studies of decomposer fungus invertebrate interactions? *Soil Biol. Biochem.* 78, 274–283 (2014).
37. A’Bear, A. D., Boddy, L., Kandeler, E., Ruess, L. and Jones, T. H. Effects of isopod population density on woodland decomposer microbial community function. *Soil Biol. Biochem.* 77, 112–120 (2014).
38. Sheppard, S. K. et al. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Mol. Ecol.* 14, 4461–8 (2005).
39. Paula, D. P. et al. Detection and decay rates of prey and prey symbionts in the gut of a predator through metagenomics. *Mol. Ecol. Resour.* n/a–n/a (2015). doi:10.1111/1755-0998.12364
40. Varennes, Y.-D., Boyer, S. and Wratten, S. D. Un-nesting DNA Russian dolls - The potential for constructing food webs using residual DNA in empty aphid mummies. *Mol. Ecol.* 3925–3933 (2014). doi:10.1111/mec.12633
41. Falk, D. D., Palmer, M. A. and B, Z. J. *Foundations of Restoration Ecology.* (Island Press, 2006).
42. Lefcheck, J. S. and Duffy, J. E. Multitrophic Functional Diversity Predicts Ecosystem Functioning in Experimental Assemblages of Estuarine Consumers. *Ecology* 150523083517004 (2015). doi:10.1890/14-1977.1
43. Boyer, S. Fauna in decline: The community way. *Science* (80-.). 346, 821–821 (2014).

Author contributions statement

SB conceived the manuscript and prepared the figures. SB, BRW and SDW edited the manuscript

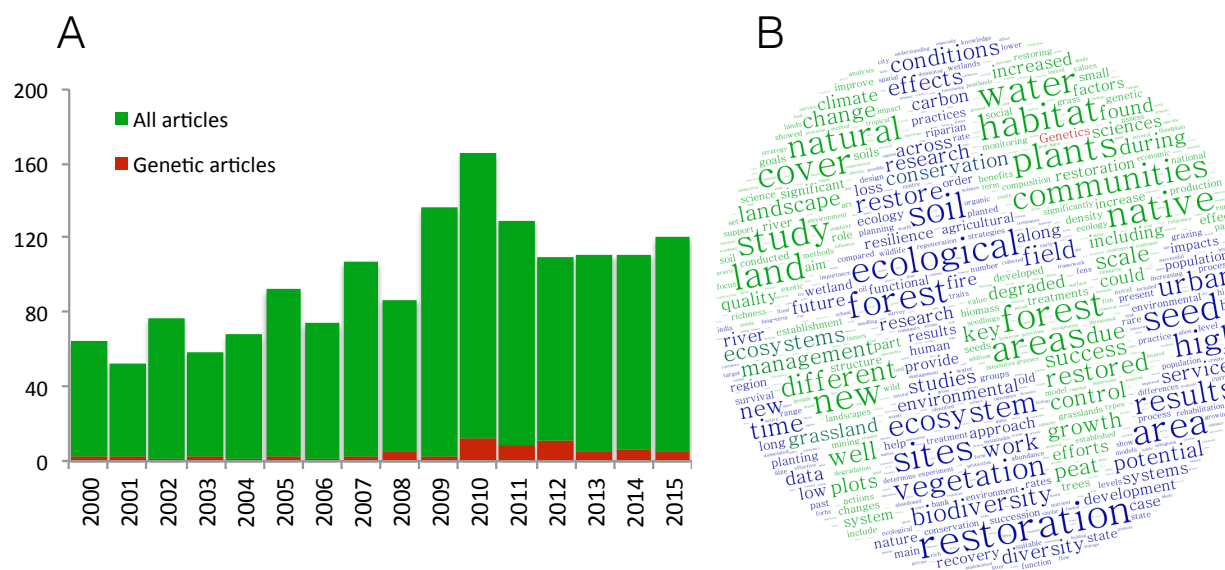


Figure 1. Limited presence of genetic studies in restoration ecology publications (A) and conference presentations (B). A: Number of articles published in the journal Restoration Ecology between 2000 and 2015 according to a search on Web Of Knowledge using the following criteria: Journal: Restoration Ecology (in green) ; Journal: Restoration Ecology AND Topic: DNA OR RNA OR Genetic OR Genomic OR Genotype (in red). B: Visual summary of the book of abstract (oral presentations only) from the 2015 Restoration Ecology conference in Manchester (SER 2015). The size of the words reflects the number of times they appear in the book of abstract. In red, the word genetic, which appeared in 21 abstracts out of 328. Other colours are for illustrative purpose only. Common English words, and words relating to authors names and addresses were removed

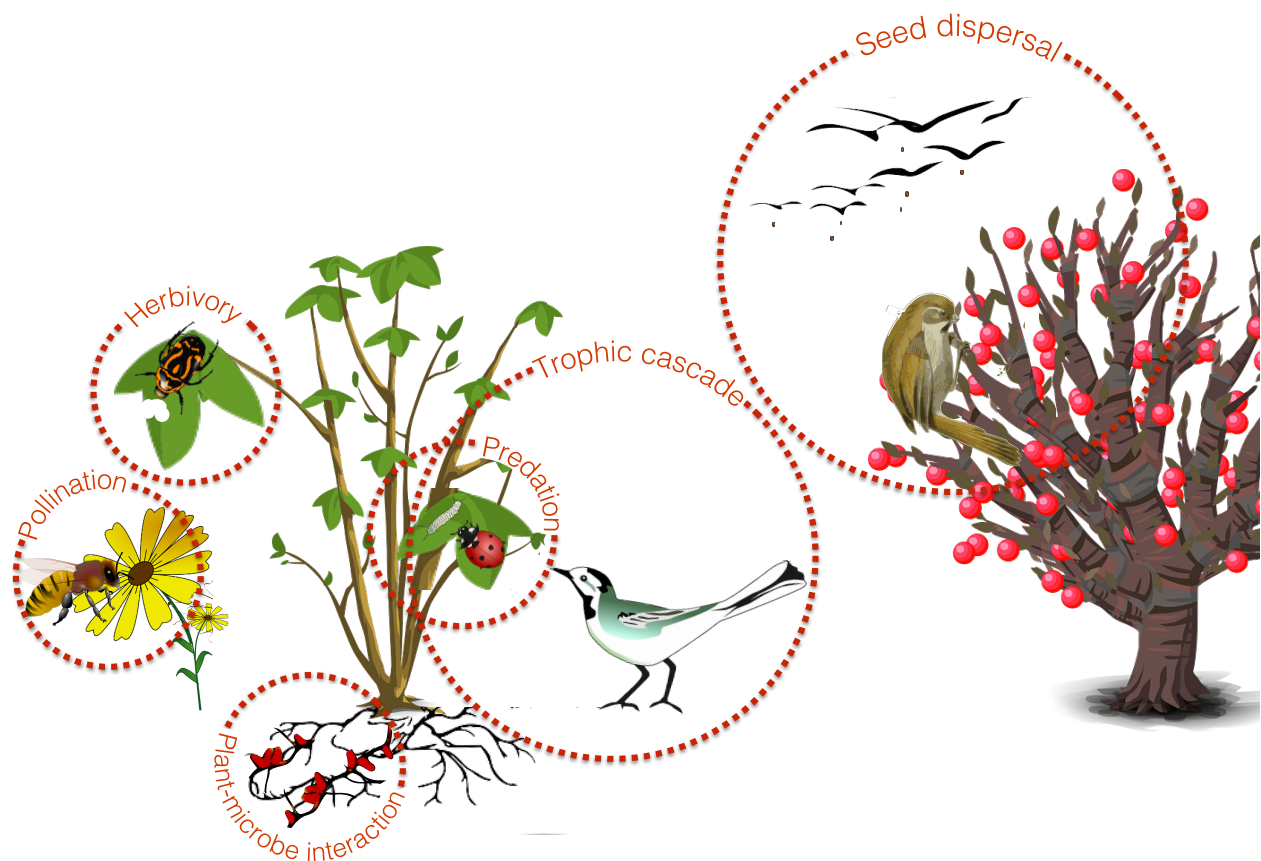


Figure 2. Main types of interspecific interactions for which molecular approaches can better inform ecological restoration projects.