

5. Ward, J.D. (2015) Rapid and precise engineering of the *Caenorhabditis elegans* genome with lethal mutation co-conversion and inactivation of NHEJ repair. *Genetics* 199, 363–377
6. Zhao, P. *et al.* (2014) Oligonucleotide-based targeted gene editing in *C. elegans* via the CRISPR/Cas9 system. *Cell Res.* 24, 247–250
7. Dickinson, D.J. and Goldstein, B. (2016) CRISPR-based methods for *Caenorhabditis elegans* genome engineering. *Genetics* 202, 885–901
8. Arribere, J.A. *et al.* (2014) Efficient marker-free recovery of custom genetic modifications with CRISPR/Cas9 in *Caenorhabditis elegans*. *Genetics* 198, 837–846
9. Dickinson, D.J. *et al.* (2015) Streamlined genome engineering with a self-excising drug selection cassette. *Genetics* 200, 1035–1049
10. Champer, J. *et al.* (2016) Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nat. Rev. Genet.* 17, 146–159

Forum

Parasites Lost: Neglecting a Crucial Element in De-Extinction

Christian Selbach,^{1,*}
Philip J. Seddon,¹ and
Robert Poulin¹

Bringing back iconic and beloved extinct species is a hot and intensely debated current topic. Yet, the parasites of de-extinction candidate species have remained largely overlooked in this debate. Here we point out the potentially far-reaching ecological impacts of bringing back extinct species without their parasites.

De-extinction is the resurrection of extinct species, or ecological proxies that closely resemble them, using selective breeding, cloning, or cutting-edge genetic engineering techniques [1]. The most prominent de-extinction candidates include long-gone but iconic animals, such as the woolly mammoth (*Mammuthus primigenius*), or more recently lost charismatic

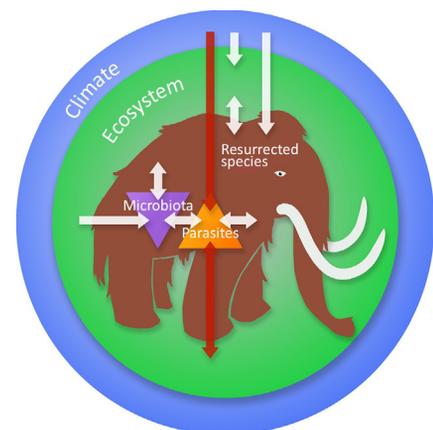
mammals and birds, such as the Tasmanian tiger (*Thylacinus cynocephalus*), and the passenger pigeon (*Ectopistes migratorius*), with the aim of restoring lost ecosystem functions and processes through the establishment of free-ranging populations [2]. As more and more technical difficulties are being overcome, such projects have moved from science fiction to real plausibility [3]. Consequently, de-extinction is a hot current topic and its feasibility, costs, ecological value, and risks, as well as legal status, are intensely debated (e.g., [4]).

One issue that has been largely overlooked in this debate is the role of parasites that went coextinct with their host, but of which we have little or no knowledge, and no means of (or often no interest in) resurrecting. Although operating in time rather than space, de-extinction is largely an issue of conservation translocation, a practice already well established in conservation biology and aiming at establishing species within a suitable habitat [2]. The importance of parasites is slowly being recognized in conservation biology (e.g., [5]). In contrast to reintroduced extant species however, resurrected species would likely come back with few of their original parasites still being present. Apart from brief mentions [3,6], the ecological implications of parasite absence have not featured in the current de-extinction discussions, despite the many important structuring roles parasites play in ecosystem functions, such as shaping and maintaining host population dynamics, playing a central role in food-web connectivity, or functioning as ecosystem engineers.

It is important to include host–parasite interactions as well as interactions between parasites and other organisms when considering de-extinction projects and the assessment of their potential ecological impacts (Figure 1). Here, we use a deliberately broad definition of parasites,

referring to macroparasites, such as helminths and arthropods, as well as microparasites, including parasitic protozoans, bacteria, and viruses, since they could all interact with resurrected species. This clearly distinguishes parasites from symbiotic microbiota, such as the gut microbiota. While the latter are seen as crucial elements of successful resurrection processes, parasites are usually regarded as threats to, rather than integral parts of, organisms and ecosystems in this context, and are considered only when critical to the restoration of the host species [6].

In order to explore what it would mean to have resurrected species (initially) free of parasites returned to an ecosystem, we can outline possible scenarios of the interaction of resurrected species and parasites by drawing analogies and giving examples from biological invasion processes and species (re-)introductions. These scenarios include case studies of parasite/enemy-release, parasite dilution, spill-over, and spill-back situations and their potential impacts on resurrected and extant species, and whole ecosystems (Table 1). The ecological and



Trends in Parasitology

Figure 1. The Main Interactions between a Resurrected Species, Its Environment, Microbiota, and Parasites. Directions of interactions are indicated by arrows; previously neglected interactions that are discussed here are highlighted in red. Modified from [6], with permission.

Table 1. Possible Scenarios of the Interaction between Resurrected Species and Parasites, Their Potential Ecological Impacts, and Case Studies from Biological Invasions and Reintroductions

	Scenario	Possible outcome	Ecological impact	Case studies	Refs
1.	All extinct parasites brought back with their host	Fully functional host–parasite system resurrected; parasite spill-over of newly resurrected parasites to extant species possible	<ul style="list-style-type: none"> Parasite spill-over to extant species with potential detrimental effects 	–	
2.	No/limited parasite acquisition from extant species	Resurrected species remains parasite-free or has lower parasite loads than originally; might initially do well and could even ‘outperform’ their original ancestors under parasite-release	<ul style="list-style-type: none"> Could outcompete and displace extant groups Possible invasion into other habitats Uncertain long-term effects on host (e.g., immunology) 	Parasite release in invasive species: <ul style="list-style-type: none"> European green crab (<i>Carcinus maenas</i>) European starlings (<i>Sturnis vulgaris</i>) have lower parasite diversity in North America Australian cane toad (<i>Rhinella marina</i>) 	[10] [10] [9]
3.	Parasite acquisition from extant species	Resurrected species could interfere with extant parasite transmission and lower the parasite burden of the extant host (dilution effect by acting as sink/decoy, dead-end host)	<ul style="list-style-type: none"> Extant species might benefit from a release in parasite burdens 	Invasive species leading to parasite dilution: <ul style="list-style-type: none"> Introduced brown trout (<i>Salmo trutta</i>) act as parasite sink in New Zealand, potential dilution effect Australian cane toad (<i>Rhinella marina</i>) 	[11] [9]
		Resurrected species can be competent reservoir hosts (or vectors) for extant parasites and could amplify transmission dynamics of parasite populations, e.g., via parasite spill-back	<ul style="list-style-type: none"> Effect might go unnoticed for some time, since these parasites are normal in ecosystem Potentially negative effects; could threaten extant species 	Parasite spill-back from invasive species: <ul style="list-style-type: none"> European starlings (<i>Sturnis vulgaris</i>) Introduced salmonid harbour native acanthocephalan in Argentina Displacement of native house gecko (<i>Lepidodactylus lugubris</i>) via parasite spill-back Australian cane toad (<i>Rhinella marina</i>) 	[8] [8] [8] [9]
		Resurrected species cannot cope with acquired parasites and goes re-extinct (due to lack of coevolution and low host resilience)	<ul style="list-style-type: none"> Possibly no negative impact on ecosystem De-extinction project fails 	–	
		Resurrected populations acquire parasites without major dilution, spill-back or extinction events; parasites may play a small role in regulating host populations	<ul style="list-style-type: none"> Possibly no negative impact on ecosystem 	Regulating role of parasites on re-introduced species: <ul style="list-style-type: none"> Grey wolves (<i>Canis lupus</i>) in Yellowstone National Park 	[12]

evolutionary impacts and the mechanisms behind these processes have recently been reviewed in the context of biological invasions in aquatic ecosystems (e.g., [7]), but would equally apply to resurrected species in terrestrial systems.

What is likely to happen in the case of a successful species resurrection depends on a multitude of factors, ranging from the choice of candidate (whether parasites from closely related extant hosts are present; solitary vs. social life style; high vs. low population densities), to the environment in which it is released (e.g., free-range vs. enclosures), and the way it is

managed (e.g., food supplementation; veterinary care; culling). Even if bringing back extinct parasites was technically possible in cases where we have adequate samples – such as ectoparasites from museum specimens of plumage or pelt, or endoparasite DNA found in coprolithes or preserved faeces – it seems rather unlikely that resources would be spent doing so, especially since parasites are not as iconic, beloved, and missed [2] as their free-living hosts. However, since a parasite-free individual in itself constitutes an ecosystem with unfilled niches, it is inevitable that these positions will be filled eventually. Just as invasive species acquire new parasites [8], resurrected

species can be expected to be competent hosts for a range of extant parasites, with possible outcomes described in Table 1.

Different scenarios could also occur in succession. For example, no parasite acquisition might occur for a period of time, allowing the resurrected species to prosper under parasite-release, followed by later acquisition events and possible shifts in ecological impacts. Likewise, some outcomes are not mutually exclusive and could happen simultaneously. For example, resurrected species might act as a sink and diluter for one parasite species and negatively affect

its transmission dynamics, while also amplifying the transmission of another species via spill-back. Such a dynamic host–parasite interaction has been described for invasive species, such as the cane toad (*Rhinella marina*) in Australia [9]. Initially introduced for pest control in the 1930s, cane toads left many of their native parasites and pathogens behind and were able to rapidly spread through the country, where they acquired and spread new parasites (e.g., the invasive pentastome *Raillietiella frenata*) that can spill back to native hosts. At the same time, cane toads represent dead-end hosts for other native parasites and can reduce parasite loads in native host taxa, and even lead to the decline of parasite species [9]. Although the exact impact of resurrected species on parasite dynamics can only be speculative, it can be expected that these organisms would not remain parasite-free for long and are likely to interact with extant host–parasite systems, just as invasive species do. Especially hyper-abundant and highly mobile candidates, such as the passenger pigeon (*Ectopistes migratorius*), could have the potential to act as migrating vectors of parasites and pathogens. Furthermore, host–parasite relationships are not static, and environmental factors, such as climate change, can have far-

reaching effects on parasites and their transmission dynamics, with often hard-to-predict ecological outcomes. Such changes would also affect the acquired parasite fauna of resurrected species, once released into their environment (Figure 1).

Rapidly developing technological advances will make de-extinction, or proxy species creation, possible in the not-too-distant future [3]. Besides the moral questions this raises, the ecological benefits and associated risks need to be carefully assessed. Since resurrected animals (or their ecological proxies) constitute hosts for parasites, it is essential to include parasites in any assessment of the potential ecological impacts of de-extinction projects. Altogether, this highlights the complexity of introducing ecological proxies into an ecosystem.

Acknowledgments

We are grateful to Helen Taylor for her input, and to Jonas Heidebrecht for help with the figure. We also thank the two anonymous referees for their insightful comments that greatly improved the manuscript. CS received a postdoctoral fellowship from the German Research Foundation (DFG, SE 2728/1-1).

¹Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

*Correspondence:

christian.selbach@otago.ac.nz (C. Selbach).
<https://doi.org/10.1016/j.pt.2017.08.003>

References

- Shapiro, B. (2017) Pathways to de-extinction: how close can we get to resurrection of an extinct species? *Funct. Ecol.* 31, 996–1002
- Seddon, P.J. *et al.* (2014) Reintroducing resurrected species: selecting DeExtinction candidates. *Trends Ecol. Evol.* 29, 140–147
- IUCN SSC (2016) IUCN SSC Guiding Principles on Creating Proxies of Extinct Species for Conservation Benefit, Version 1.0. IUCN Species Survival Commission.
- Bennett, J. *et al.* (2017) Spending limited resources on de-extinction could lead to net biodiversity loss. *Nat. Ecol. Evol.* 1, 0053
- Spencer, H.G. and Zuk, M. (2016) For host's sake: the pluses of parasite preservation. *Trends Ecol. Evol.* 31, 341–343
- Wood, J.R. *et al.* (2017) Using palaeoecology to determine baseline ecological requirements and interaction networks for de-extinction candidate species. *Funct. Ecol.* 31, 1012–1020
- Goedknecht, M.A. *et al.* (2016) Parasites and marine invasions: ecological and evolutionary perspectives. *J. Sea Res.* 113, 11–27
- Kelly, D.W. *et al.* (2009) Parasite spillback: a neglected concept in invasion ecology? *Ecology* 90, 2047–2056
- Selechnik, D. *et al.* (2016) The things they carried: the pathogenic effects of old and new parasites following the intercontinental invasion of the Australian cane toad (*Rhinella marina*). *Int. J. Parasitol. Parasites Wildl.* Published online December 29, 2016. <http://dx.doi.org/10.1016/j.ijppaw.2016.12.001>
- Torchin, M.E. *et al.* (2003) Introduced species and their missing parasites. *Nature* 421, 628–630
- Poulin, R. *et al.* (2011) Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biol.* 56, 676–688
- Almberg, E.S. *et al.* (2012) Parasite invasion following host reintroduction: a case study of Yellowstone's wolves. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 367, 2840–2851