

Box 2. A comparative approach to further derive process from phylogenetic patterns of *Wolbachia* distribution. While much previous research has provided in depth understanding of the distributions of *Wolbachia* among arthropods as well as identifying many underlying processes, we outline a program of comparative (i.e., multi-system) future research that should be undertaken to definitively evaluate our propositions with specific reference to CI-inducing *Wolbachia* strains. Research programs should be conceived that focus on building a better understanding among other arthropod taxa comprising the majority of species where community composition cannot be easily recorded within host-specific, phenologically synchronised plant tissues such as is found among fig wasps.

Laboratory experiments: A first step should focus on the experimental manipulation of infection status (e.g., with antibiotic curing) to further validate or contest the physiological mechanisms proposed. Such data can confirm whether a system of study that is yielding high predictive accuracy from WOLPREDICTOR evaluation actually contains CI-inducing *Wolbachia* strains. Second, the *Wolbachia* CI factor (*cif*) genes responsible for both causing reproductive incompatibility (i.e., embryo killing) and the paired curing of CI through sperm modification rescue (e.g., paired *cifA* and *cifB* genes encoded in the prophage WO) should be sequenced. Here, the identification of compatible-incompatible specific sequence variants would provide further demonstration that CI was indeed acting in this system (Bordenstein & Bordenstein 2016; LePage *et al.* 2017; Shropshire *et al.* 2018; Beckmann *et al.* 2019). Finally, and with particular reference to the oviposition trade-off hypothesis, experiments should be conducted to ascertain whether post-zygotic fecundity losses in fig wasps (or other taxa) are enacted through mechanisms (e.g., egg senescence, reduced sperm fitness, selective oviposition) that engender favourable exploitation of reproductive resources (e.g., fig wasp oviposition sites).

Sampling strategies and field experiments: It should be a priority for ambient insect trapping experiments to routinely and systematically record associated environmental trapping metadata. These may include, altitude, abiotic conditions, habitat type, geographic distances between sampling sites, phylogenetic composition of the sampling site, and knowledge of the wider insect community associated with each host. Moreover, insect communities should be sampled with a methodological and targeted focus on host plant trap placement (e.g., in physical proximity of species of congeneric co-occurring plants) in order to evaluate through the construction of food-webs and endosymbiont assay whether systemic patterns of *Wolbachia* infection become apparent. Through these methods, the distributions of *Wolbachia* relative to the associated levels of species diversity (e.g., through basic barcoding methods; Hebert *et al.* 2003) can be used to inform hypothetical incidences/intensity of ecological contact and community membership. These data can then be formatted for WOLPREDICTOR to generate predictive accuracy outcomes for species and communities under investigation. Existing insect samples and datasets collected in a community context are available as immediate candidate systems for *Wolbachia* screening. In general, future studies seeking to resolve insect-plant food webs should consider the collection and screening of individuals for *Wolbachia* and other endosymbionts as standard procedure.

Alternative genomic mechanisms: WOLPREDICTOR could easily be configured to investigate other mechanisms of ephemeral (or trivial) RI among taxa that do not possess, potentially due to contingencies determined by genomic architecture, CI-inducing *Wolbachia*. For example, *Wolbachia* may be particularly suited to haplodiploidy while other mechanism may dominate among different sex-determination systems. Here we suggest that genomic inversions may be prevalent among the X-Y sex chromosome system in Diptera (Noor *et al.* 2001; Noor & Bennett 2009), or that single point mutation shell chirality reversal may operate among gastropods (Hoso *et al.* 2010). Investigation of genomic conditions may permit the categorisation of RI types among focal species equivalent to our demarcation of *wsp* clades as compatible/incompatible determinants between mating partners as data levels in WOLPREDICTOR.

Summary: We suggest screening for systemic distributions of *Wolbachia* i) across species in close ecological contact (candidates for horizontal transmission) and ii) across individuals that share habitat for mating and breeding sites (candidates for vertical transmission), while testing for genomic and physiological corroborating factors.