7-22-2020

Insights from Population Genomics to Enhance and Sustain Biological Control of Insect Pests

Arun Sethuraman  
*California State University San Marcos*

Fredric J. Janzen  
*Iowa State University*

David W. Weisrock  
*University of Kentucky, david.weisrock@uky.edu*

John J. Obrycki  
*University of Kentucky, john.obrycki@uky.edu*

Follow this and additional works at: [https://uknowledge.uky.edu/biology_facpub](https://uknowledge.uky.edu/biology_facpub)

Part of the *Biology Commons*, and the *Entomology Commons*

Right click to open a feedback form in a new tab to let us know how this document benefits you.

**Repository Citation**

Sethuraman, Arun; Janzen, Fredric J.; Weisrock, David W.; and Obrycki, John J., "Insights from Population Genomics to Enhance and Sustain Biological Control of Insect Pests" (2020). *Biology Faculty Publications*. 192.  
[https://uknowledge.uky.edu/biology_facpub/192](https://uknowledge.uky.edu/biology_facpub/192)

This Review is brought to you for free and open access by the Biology at UKnowledge. It has been accepted for inclusion in Biology Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Insights from Population Genomics to Enhance and Sustain Biological Control of Insect Pests

Notes/Citation Information
Published in Insects, v. 11, issue 8, 462.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Digital Object Identifier (DOI)
https://doi.org/10.3390/insects11080462

This review is available at UKnowledge: https://uknowledge.uky.edu/biology_facpub/192
Insights from Population Genomics to Enhance and Sustain Biological Control of Insect Pests

Arun Sethuraman 1,*, Fredric J. Janzen 2,3, David W. Weisrock 4 and John J. Obrycki 5

1 Department of Biological Sciences, California State University San Marcos, San Marcos, CA 92096, USA
2 Department of Ecology, Evolution, & Organismal Biology, Iowa State University, Ames, IA 50010, USA; fjanzen@iastate.edu
3 Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, USA
4 Department of Biology, University of Kentucky, Lexington, KY 40506, USA; david.weisrock@uky.edu
5 Department of Entomology, University of Kentucky, Lexington, KY 40506, USA; john.obrycki@uky.edu

* Correspondence: asethuraman@csusm.edu

Received: 18 June 2020; Accepted: 17 July 2020; Published: 22 July 2020

Abstract: Biological control—the use of organisms (e.g., nematodes, arthropods, bacteria, fungi, viruses) for the suppression of insect pest species—is a well-established, ecologically sound and economically profitable tactic for crop protection. This approach has served as a sustainable solution for many insect pest problems for over a century in North America. However, all pest management tactics have associated risks. Specifically, the ecological non-target effects of biological control have been examined in numerous systems. In contrast, the need to understand the short- and long-term evolutionary consequences of human-mediated manipulation of biological control organisms for importation, augmentation and conservation biological control has only recently been acknowledged. Particularly, population genomics presents exceptional opportunities to study adaptive evolution and invasiveness of pests and biological control organisms. Population genomics also provides insights into (1) long-term biological consequences of releases, (2) the ecological success and sustainability of this pest management tactic and (3) non-target effects on native species, populations and ecosystems. Recent advances in genomic sequencing technology and model-based statistical methods to analyze population-scale genomic data provide a much needed impetus for biological control programs to benefit by incorporating a consideration of evolutionary consequences. Here, we review current technology and methods in population genomics and their applications to biological control and include basic guidelines for biological control researchers for implementing genomic technology and statistical modeling.

Keywords: population genomics; biological control; demographic models; pest management

1. Introduction

Biological control—the use of natural enemies or biological control organisms such as terrestrial arthropods, microorganisms and invertebrates (e.g., entomophagous nematodes) to suppress populations of agricultural pests—has been a successful pest management tactic for over a century [1–3]. Motivated by the abundance of naturally occurring predator-prey and parasitoid-host species interactions, biological control provides benefits for pest suppression. Such benefits include the potential for long-term pest suppression and increased environmental and human safety, in comparison to the use of chemical insecticides [4]. Examples of highly successful and sustainable biological control include programs for the ash whitefly, cereal leaf beetle, alfalfa weevil and the cassava mealybug [5–8] and for additional examples see References [3,9].

However, human-mediated release of biological control organisms may have short- and long-term consequences for the evolution of (a) prey/hosts (also called ‘target’ effects), as well as (b)
released populations of biological control organisms, (c) native (resident) populations, that may
compete with released biological control organisms and (d) associated endosymbiont/microbial
diversity (collectively termed as ‘non-target’ effects) which could detrimentally affect other species
that interact with the biological control organisms [10]. At the ecological level, both target and
non-target effects of biological control have been studied broadly in the context of efficacy and
efficiency of control strategies [3]. Such examples include species interactions and resource
competition [11], host-pathogen interactions and interactions of biological control organisms with
endosymbionts and transgenic host plants [12]. Research to improve biological control programs, even
to push a 10% increase in success of importation and augmentation, continues to be a challenge [13].

With the advent of modern sequencing technologies and statistical methods to analyze
large-scale genetic data, agriculturalists and geneticists are increasingly applying population
genomics as a means to enhance our understanding of the evolution of biological control organisms
and insect pests [14–16]. Such a strategy is mindful of not just the immediate consequences of
introducing biological control organisms for pest suppression but of long-term evolutionary
trajectories of both the pest and biological control species [17]. Genomic data can offer uniquely
valuable insights into changes in population size, natural and artificial selection, migration or
admixture, inbreeding and even co-evolution of biological control organisms and their pest targets.
Population genomics hence provides an efficient means of monitoring these important factors for
success of biological control programs. Studying biological control organisms also presents a unique
and controlled opportunity to address fundamental questions about adaptive evolution,
invasiveness and co-evolution.

This review focuses on a range of fundamental issues that have been addressed using
population genomics in general but have yet to be applied to gain a better understanding of
biological control. We first summarize different methods of biological control and population
genetic models that can be used to describe them. We then focus on four core issues involving
population genomics and biological control—(1) population size change, (2) natural selection and
adaptive evolution in novel environments, (3) gene flow and (4) inbreeding. Finally, we provide
recommendations and an outline of suggested steps (a ‘pipeline’) for researchers to facilitate use of
available genomics methods to assess biological control. The emphasis of this review is on
entomophagous species, that is, predators and parasitoids that attack insect pests.

2. Application of Population Genomic Models to Biological Control

Biological control of insect pests can be classified broadly into three methods, based on the
mode(s) of manipulation of biological control organisms—importation, augmentation and
conservation. In this review, we discuss importation and augmentation, the two methods in which
arthropod biological control organisms are released into the environment. Most introduction
histories of entomophagous species are complex sequences of demographic events. These sequences
of events in turn determine current genomic diversity, population densities, sustainability and thus
success of biological control. Also, although detailed historical introduction records have been
maintained for many species of biological control organisms [18]—specific example, the predatory
lady beetle, Coccinella septempunctata [19]—the quality of data for many species is highly variable.
This is especially true for some species of insect predators that have become invasive [20,21].
However, their post-importation and augmentation history can be inferred using population genetic
models. These models represent how populations grow or decline in numbers, evolve, exchange
genes and diverge. Here we discuss biological control scenarios and population genetic models that
can be used to infer post-introductory evolutionary histories.

(a) Importation biological control is defined as the introduction of biological control organisms
in a single or repeated pulse(s) into a previously unoccupied environment [4]. Examples of
successful importation include the vedalia beetle, Rodolia cardinalis [22] and many species of insect
parasitoids [4,6]. Importation can be modeled using a “serial-founder” model [23], Figure 1A).
Figure 1. Population genetic models that are used to describe importation and augmentation of biological control organisms—(A) Serial founder model, often used to describe importation of biological control organisms, (B) Source-Sink model to describe augmentation, (C) Stepping stone model to describe establishment of new populations post-importation or augmentation, and (D) Population Growth and Bottleneck models to describe successful establishment or failure of importation and augmentation.

Serial founding of biological control organisms can occur naturally due to invasiveness or be anthropogenically mediated due to importation. Examples of serially founded biological control organism populations include an egg parasitoid (Trissolcus japonicus) of an introduced insect pest species, Halyomorpha halys [24], the Harlequin lady beetle, Harmonia axyridis [20,25] and the seven-spotted lady beetle, Coccinella septempunctata [19]. Serial founder models allow the estimation of numerous parameters, including times of serial founding of each population, genetic diversity and effective population sizes of the source and serially founded populations. Effective population sizes are different from census sizes, being more informative of the degree of genetic diversity within imported populations (see Box 1). Comparing effective population sizes of imported populations thus aids in understanding the degree of random genetic drift versus natural selection in driving their evolutionary dynamics. For example, Calfee et al. [26] compared genetic diversity of Africanized honey bees, Apis mellifera scutellata, in hybrid zones in North and South America and found no significant reduction in genetic diversity due to bottlenecks and rapid expansion. They combine these findings with a study of differential fitness, showing that natural selection has played a role in maintaining high genetic diversity in hybrid bees.

Serial founding can also incorporate gene flow between one or more founded populations to estimate migration rates and admixture parameters (see Box 1). This model further allows the estimation of “bridgehead” effects [20], which often lead to successful invasion and establishment of imported organisms in new environments.
### Box 1. Definitions of population genomic terms used in this article.

| Term                          | Definition                                                                                                                                                                                                 |
|-------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Effective Population Size** (Ne) | The size of the population that is evolving neutrally due to random genetic drift. In a randomly mating population of constant size and in the absence of natural selection, this Ne should be equivalent to the census size, Nc. The Ne of a population is often approximated as a measure of its genetic diversity. |
| **Census Population Size** (Nc) | The number of individuals in a population of a species. Changes in the census size (e.g., due to competition from congenics, insecticide use) will also affect the rate of evolution by genetic drift and therefore the population’s effective population size, Ne. Nc is difficult to measure in nature, especially in natural enemies. |
| **Natural Selection**          | Changes in allele frequencies in a population due to differential fitness of alleles or combinations of alleles.                                                                                         |
| **Genetic Drift**             | Fluctuation in allele frequencies in a population due to random sampling of alleles from one generation to the next.                                                                                     |
| **Bottleneck**                | Decrease in the census size, Nc of a population, owing to importation or augmentation, leading to a decrease in its effective population size, Ne.                                                        |
| **Genetic Diversity**         | The diversity of alleles across genomic loci in a population (allelic richness) or the average heterozygosity across genomic loci. Genetic diversity of a population is directly affected by is Nc (and therefore Ne), mating processes (random versus non-random mating), geographical population structure and natural selection. |
| **Hybrid Vigor**              | Increased fitness of hybrid strains. In natural enemies, this could be quantified as increased fecundity, mating success, range expansion and invasiveness, competition success, resource utility. |
| **Deleterious Mutations**     | Alleles that confer lower absolute fitness and thereby lower relative fitness of genotypes that carry this allele in a population.                                                                    |
| **Adaptation**                | Survival, reproduction and viability of heritable advantageous traits due to natural selection.                                                                                                             |
| **Meiotic Recombination**     | Exchange of genetic material between maternal and paternal chromosomes during meiosis. Recombination landscape is affected by genetic drift and natural selection.                                             |
| **Sexual Selection**          | Pre-mating barrier to gene flow, owing to differential mate choice. In arthropods, this could include wing or elytral patterning, chemical cues, vocalizations and size variation.                                 |
| **Inbreeding**                | Non-random mating between close relatives within a population. Inbreeding could be opportunistic (due to geography, leading to the formation of structured populations) or due to sexual selection.           |
| **Inbreeding Depression**     | Accumulation of deleterious mutations in inbred populations, leading to decreased fitness.                                                                                                               |
| **Migration/Gene Flow/Admixture/Introgression** | Physical movement and reproduction (therefore recombination) of migrant individuals from one population into another.                                                                                           |
| **Genetic Linkage**           | Co-inheritance of collinear segments of DNA owing to reduced recombination between them.                                                                                                            |
| **Linked Selection**          | Co-inheritance of non-recombinant segments of DNA due to natural selection on a linked genetic locus.                                                                                                      |
| **Genetic Hitchhiking**       | Process of co-inheritance of variants in non-recombinant segments of DNA due to positive natural selection on a linked genetic locus.                                                                     |
| **Selective Sweep**           | Pattern of reduced genetic diversity in non-recombinant segments due to genetic hitchhiking.                                                                                                             |
| **Quantitative Trait Loci**   | Genomic loci that control variability in quantitative phenotypes. Interaction across variants at different genomic loci, contributing towards variability in a trait.                                           |
| **Epistasis**                 | The average number of times every single nucleotide has been sequenced.                                                                                                                                     |
| **Sequencing Depth/Coverage** | A contiguous piece of DNA that is obtained from a sequencer, that have to be assembled to form contigs or often chromosome-size scaffolds.                                                                 |
| **SNP’s**                     | Single Nucleotide Polymorphisms - variants at a single nucleotide locus.                                                                                                                                    |

(b) Augmentation biological control embodies biological control organisms that were originally imported but failed to persist in their new environment and have their populations augmented through repeated releases, typically annually [27]. Examples of augmented biological control organisms include the greenhouse whitefly parasitoid, *Encasia formosa* and egg parasitoids.
in the genus *Trichogramma* [6,28], the mealybug destroyer, *Cryptolaemus montrouzieri* and over 230 commercially available arthropod species [29,30]. Arthropod biological control organisms from a stock population (often purchased *en masse*) can also be repeatedly introduced into an environment where they have already been established (Figure 1B) and can be modeled using a “source-sink” model. Under a source-sink model, demographic parameters such as effective population sizes of the founding source population and the recipient introduced populations and continued rates of unidirectional migration from the source to the sink population (in number or proportion of individuals per generation), can be estimated.

Population genetic models can describe aspects of biological control:

(a) Successful biological control programs can result in the establishment of introduced populations over a broad geographic range, sometimes through non-anthropogenic assisted range expansions. Examples of this process have been noted in the literature, including parasitoid Aphelinidae and Braconidae hymenopterans [31,32], the flower head weevil, *Rhinocyllus conicus* [33] and numerous invasive species (summarized in Reference [34]). This scenario can be modeled using an isolation by distance framework ([35,36], Figure 1C). Under this model, gene flow restricted to geographically proximal populations leads to increased genetic differentiation across the range of the introduced species (Figure 1C). Recent advances in utilizing genomic surveys to inform isolation by distance [37] could potentially be applied to long-range dispersal of organisms to infer fine-scale patterns of range expansions.

(b) Introduced populations of biological control organisms are often small. Thus their successful establishment depends on numerous factors, including adaptability to local environments, availability of hosts/prey and competitors. Modeling effective population size declines are thus informative of changes in genomic diversity in introduced populations and of potential utility in conservation biological control. Alternatively, unsuccessful introductions summarized in References [33,38], are also characterized by declining population sizes. Population size declines are often modeled using a bottleneck model for inbred, small populations [39,40], Figure 1D. Models incorporating population size change can estimate population growth or decline rates, along with effective population sizes of founder and introduced populations of biological control organisms. These factors can be used in tracing evolutionary trajectories and effectiveness of biological control (see discussion).

Importantly, numerous statistical methods use population genomic data to rigorously identify the best-fitting demographic model for a particular biological control system (see Table 1). Furthermore, these methods allow for the estimation of evolutionary parameters of specific interest to biological control (population size, rate of growth or decline, migration, etc.).
Table 1. List of commonly used population genomics tools for estimating evolutionary history under a variety of models.

| Software      | Statistical Method | Citation                                                                 | Purpose                                                                                   | Availability |
|---------------|--------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|--------------|
| STRUCTURE     | Bayesian MCMC      | Pritchard et al., 2000 [41]                                              | Estimating admixture proportions, ancestral subpopulation allele frequencies.               | OS, Binaries |
| PSMIX         | ML                 | Wu et al., 2006 [42]                                                    | Estimating admixture proportions, ancestral subpopulation allele frequencies.               | OS, R package|
| ADMIXTURE     | ML                 | Alexander et al., 2009 [43]                                             | Estimating admixture proportions, ancestral subpopulation allele frequencies.               | Binary only  |
| FRAPPE        | ML                 | Tang et al., 2005 [44]                                                  | Estimating admixture proportions, ancestral subpopulation allele frequencies.               | Binary only  |
| EIGENSTRAT    | PCA                | Price et al., 2006 [45]                                                 | Estimating population stratification                                                     | OS, Binaries |
| IM            | Bayesian MCMC      | Hey and Nielsen 2004 [46]                                               | Estimating ancestral demography under an Isolation with migration model                   | OS, Binaries |
| IMA2          | Bayesian MCMC      | Hey and Nielsen 2007 [47], Hey 2010 [48]                                 | Estimating ancestral demography under an Isolation with migration model                   | OS, Binaries |
| IMA2p         | Bayesian MCMC      | Sethuraman and Hey 2016 [49]                                            | Estimating ancestral demography under an Isolation with migration model                   | OS           |
| MIGRATE       | Bayesian MCMC      | Beerli and Felsenstein 2001 [50], 1999 [51], Beerli 2008 [52]           | Estimating ancestral demography under an island model                                      | OS           |
| BayesAss      | Bayesian MCMC      | Wilson and Rannala 2003 [53]                                            | Estimating recent migration under a divergence model                                      | OS, Binaries |
| MDIV          | Bayesian MCMC      | Nielsen and Wakeley 2001 [54]                                           | Estimating ancestral demography under an Isolation with migration model                   | OS, Binaries |
| LAMARC        | Bayesian MCMC      | Kuhner 2006 [55]                                                        | Estimating ancestral demography under an island model                                      | OS, Binaries |
| DIYABC        | ABC                | Cornuet et al., 2010 [56]                                               | Testing complex population histories and estimate parameters                             | OS, Binaries |
| MSVAR         | Bayesian MCMC      | Beaumont 2003 [57]                                                      | Estimating population size change under a panmictic model                                  | OS           |
| FASTSTRUCT    | ML                 | Chen et al., 2006 [58]                                                  | Estimating admixture proportions, ancestral subpopulation allele frequencies.             | Binary only  |
| BAPS          | Bayesian MCMC      | Corander et al., 2006 [59]                                              | Estimating admixture proportions, ancestral subpopulation allele frequencies.              | Binaries only|
| ADMIXTOOLS    | Summary Statistics | Patterson et al., 2012 [60]                                             | Tests of admixture occurrence                                                            | OS           |
| TREEMIX       | ML                 | Pickrell and Pritchard 2012 [61]                                        | Inferring divergence and mixtures from genomic data                                        | OS           |
| FLUCTUATE     | Bayesian MCMC      | Kuhner, Yamato and Felsenstein 1998 [62]                                | Inferring population size change from genetic data                                        | OS           |
| BOTTLENECK    | Bayesian MCMC      | Cornuet and Luikart 1996 [40]                                           | Inferring population size bottlenecks from genetic data                                  | Binary only  |
| FASTSTRUCTURE | Bayesian MCMC      | Raj et al., 2014 [63]                                                   | Inferring population structure from SNP data                                            | OS           |
| GPHOCS        | Bayesian MCMC      | Gronau et al., 2012 [64]                                                | Inferring demography from individual genome sequences                                    | OS           |
| PSMC          | HMM                | Li and Durbin 2010 [65]                                                 | Inferring population size history from diploid genomes                                   | OS           |
| FASTSIMCOAL2  | Bayesian MCMC,     | Excoffier et al., 2013 [66]                                             | Inferring ancestral demography from SNP data                                            | Binary only  |
| Method       | Framework | Methodology                                                                 | Publication                                | Source |
|--------------|-----------|------------------------------------------------------------------------------|-------------------------------------------|--------|
| DADI         | ML        | Inferring ancestral demography from SNP data, testing complex population histories | Gutenkunst et al., 2010 [67]              | OS     |
| ABCreg       | ABC       | Testing complex population histories and estimate parameters                 | Excoffier et al., 2009 [68]              | OS     |
| STRUCTURAMA  | Bayesian MCMC | Estimating admixture proportions, ancestral subpopulation allele frequencies. | Huelsenbeck and Andolfato 2011 [69]     | OS     |
| DICAL        | HMM       | Inferring demography from individual genome sequences                        | Sheehan et al., 2013 [70]                | OS     |
| SWEED        | ML, LLR   | Inferring selective sweeps                                                   | Pavlidis et al., 2013 [71]              | OS     |
| SWEEPFINDER2 | ML, LLR   | Inferring selective sweeps                                                   | DeGiorgio et al., 2016 [72]             | OS     |
| MLNE         | ML        | Inferring contemporary effective population size                            | Wang and Whitlock 2003 [73]             | OS     |
| LDNE         | Summary Statistics | Inferring contemporary effective population size | Do et al., 2014 [74]                        | OS     |

ML = Maximum Likelihood, MCMC = Markov Chain Monte Carlo, LLR = Likelihood Ratio Test, PCA = Principal Components Analysis, OS = Open Source.
3. Genomic Signatures during Biological Control

Post-introductory demographic history of biological control organisms can be complex to model but can be characterized by estimating four major "parameters" of populations using genomic data—population size change, adaptation, admixture or migration and inbreeding [17], (Box 1). Here we provide an overview of these parameters and discuss how they can be estimated from genomic data derived from organisms released for biological control.

3.1. Population Size Change

Bottlenecks and change in effective population sizes both influence genomic diversity of species. Species utilized for biological control are subject to both these processes, depending on their natural history and interactions. Newly introduced populations of biological control organisms often undergo bottlenecks, where a relatively small sample of founder individuals from a larger population is introduced into a novel environment [17,75–78]. Conversely, population size growth can be enhanced in introduced populations via “invasiveness” or the uncontrolled growth of a population in a non-native (introduced) environment (e.g., Harmonia axyridis—[79]. Invasiveness of biological control organisms could be primarily due to plastic phenotypic response to changing environments [80], hybrid vigor [26,81] or rapid life-history evolution [82]. Expanding (and invading) populations evolve faster, owing to increased efficacy of selection in purging deleterious mutations and fixing advantageous ones, compared to declining or bottlenecked populations [83].

Inferring effective population sizes and changes serves as a primary indicator of population genomic processes affecting the ecological success of biological control (i.e., establishment of the biological control organisms followed by a reduction in the pest population density) and provides a much more informative alternative to otherwise detailed and labor intensive census size estimation. Applied in combination with other population genomics statistics, effective population size estimation is a means to building hypotheses to explain the success or failure of biological control programs (see Table 2).

3.2. Natural Selection and Evolution

Populations of biological control organisms in new environments, apart from undergoing population size change, are also subject to adaptive evolution in response to selection. Broadly, selection nudges populations towards fitness peaks [84].

The genetics of adaptive evolution in introduced and invasive species have been studied extensively but not in the context of biological control [21,85–88]. Numerous cases of failed introductions of biological control organisms have been noted, however, presumably owing to differential fitness [75,86,89], strong directional selection due to insecticide use [90] and sexual selection and the ‘Goldilocks principle’ [91] or adaptive evolution of traits to a selective optimum in response to environmental selection. Other factors that contribute to the success of biological control by influencing the rate of adaptive evolution of introduced individuals to the new environment include linked selection and divergence hitchhiking [92,93], migration and admixture [26,94] and inbreeding [95,96].

Multiple introductions of the same species, including populations from different geographic sources, can play a prominent role in local adaptation, invasiveness and boosting genomic diversity in populations of biological control organisms. Biological control has the distinction of having extensive introduction records over recent time scales [18,19], thus quantifying genomic variation of imported or augmented biological control organisms allows researchers and biological control administrators to study, with temporal validation, their adaptive potentials to new environments. Of particular interest are quantitative trait loci (QTLs) that contribute directly to adaptive evolution of biological control organisms in new environments. Studying the effects of natural selection on QTLs thus can be used to predict both the success or failure to establish in novel environments and
the evolutionary potential for invasiveness in biological control organisms. These data could be invaluable in informing selective breeding programs for developing more effective biological control organism populations for subsequent introduction. Most methods to detect natural selection utilize diversity and polymorphism indices across the genome and are summarized in Table 1.

3.3. Gene Flow (Admixture/Migration)

Gene flow can occur to varying extents between proximal established populations of biological control organisms and even between established populations and newly introduced populations of biological control organisms.

Ongoing gene flow between newly introduced and established populations of biological control organisms [20,97–101] indicates the absence of environmental or reproductive barriers to hybridization. This process could indicate persistence and improved fitness of hybrids of colonizing and native populations through adaptive introgression [102,103]. Conversely, reduced or even no, contemporary gene flow could occur due to geographic or genomic barriers to migration. This process could signal the presence of population structure, inbreeding and reduced genomic diversity [104].

Beyond gene flow per se, reduced fitness of hybrid populations (outbreeding depression) has been observed during reintroduction episodes [105] due to epistasis between different genomic backgrounds. Estimating population structure and gene flow from genomic data can hence be used by biological control practitioners both to understand the successful establishment of newly introduced biological control organisms and to track genomic mechanisms of successful augmentation of previously established populations, both of which are otherwise intractable via observational studies.

3.4. Inbreeding

Non-random mating of close relatives in a population reduces genetic diversity, elevates homozygosity and fixes deleterious mutations (genetic load) [94,95,106]. This inbreeding depression not only reduces population fitness but also results in population structure due to genetic drift, wherein individuals within a subpopulation are genetically more similar to each other than to individuals from other subpopulations.

Inbreeding, although widely expected during primary introductions of species for biological control, is yet to be characterized in most species at the genetic level. Some cases of inbreeding have been reported in field populations of the convergent lady beetle, *Hippodamia convergens* [98] and in the Asian lady beetle, *Harmonia axyridis* [20]. However, understanding the long-term effects of inbreeding in these and other species using genomic data remains a nascent endeavor.

Estimating inbreeding using genetic data from populations of biological control organisms in conjunction with assays of fecundity, competition and efficiency of feeding on pests can inform success of biological control programs. For example, lab-inbred (Eastern and Western USA) populations compared to outbred (augmented Eastern-Western USA hybrid) populations of *H. convergens*, lack phenotypic variability despite genetic differences and exhibit equitable success in pea aphid utilization [107]. Tools to estimate inbreeding often use summary statistics such as Identity By Descent (IBD) probabilities, inbreeding coefficients and runs of homozygosity (ROH), often only delimited by the types of genetic data used to compute them.

4. Discussion and Recommendations

4.1. Genomic Considerations for Successful Biological Control

What comprises a successful biological control program? As summarized by [108] based on more than 800 studies, primary indicators of success in biological control are reduced pest abundance and increased pest mortality, relative target versus non-target effects and the type of biological control organism - generalist (polyphagous) versus specialist. In Table 2 we develop a population genomic framework for five measures of success of biological control organisms sensu
Insects 2020, 11, 462

—(1) efficacy and establishment, measured using genetic diversity estimates; (2) spatio-temporal distribution, measured with divergence times and post-introductory evolutionary history; (3) managed breeding techniques, informed using studies of natural selection; (4) non-target effects and invasiveness, assessed via genetics of populations in imported or augmented environments; and (5) biotic effects on target/control organisms, measured using estimates of population structure, gene flow and inbreeding.

**Table 2.** Indicators of success of biological control programs and how we can measure/estimate these using population genomic methods. All methods listed either utilize microsatellite or Short Tandem Repeat (STR) markers, Single Nucleotide Polymorphisms (SNPs) or haplotype data generated from common genotyping and sequencing platforms.

| Category                              | Ecological Parameters                                      | Evolutionary Parameters                        | Genomic Method                                                                 | Evolutionary Perspective                                                                                                                                 |
|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Agent efficacy, establishment         | Mortality/survivorship, abundance before/after release     | Effective population size                       | Contemporary Ne—Colony2, ONeSamp, Estim, etc.—see Gilbert and Whitlock 2015 [109], Ancestral and current Ne—IM, IMa2, IMa2p, MIGRATE, LAMARC, PSMC | Ne measures the size of the natural enemy population evolving neutrally by genetic drift. It differs from census sizes, in that it offers a perspective on genetic diversity and hence adaptability of the population, response to new environments and resilience to failed introductions. Ancestral Ne versus current Ne thus determines increase or decrease in genomic diversity. |
| Spatio-temporal distribution          | Spatial, temporal scale assessment of abundance, distribution | Divergence times, time since population size change, phylogeography | TreeMix, IM, IMa2, IMa2p, BEAST, DIYABC, MrBayes, Bottleneck, MSVAR, FLUCTUATE, LAMARC, GeoPhyloBuilder, etc. | Broadly lumped together as genomic diversity indices, all these indices are indicators of the ‘genetic health’ of the introduced population. Successful control programs would thus expect sustainable natural enemy populations to have higher genetic diversity, polymorphism, differentiation with respect to other populations and thus lower homozygosity and inbreeding. |
| Agent management techniques           | Agent manipulation by strain selection                      | Selection, demography                           | Fst-GWAS, SweepFinder, SweeD, McDonald-Kreitman tests                         | Divergence time estimates provide evidence of time since introduction of natural enemies. Similarly, time since population size change can be used to estimate times of bottlenecks or invasiveness. Phylogeography studies also allow overlaying the current phylogenetic tree over geographical data. |

Estimating genome-wide selection across strains allows prediction of genotype-phenotype interactions and efficacy of selection in adaptive evolution of the natural population.
We propose that studies of success of biological control are essentially incomplete without a sufficient mix of manipulative experiments and genomics, which provide foundational insight into crucial ecological factors. Common denominators affect the ability of biological control organism populations to (i) establish, persist and grow in an introduced environment, (ii) withstand environmental and genomic pressures and evolve adaptively, (iii) avoid “escaping” into invasiveness and (iv), broadly, limit differential non-target effects. These factors are phenotypic differences in traits, which have underlying genomic differences within and between populations and ecological variation across geographically distinct populations of the species. Drawing on a classic example, the successful introduction of the vedalia beetle, *Rodolia cardinalis*, to suppress the cottony-cushion scale, *Icerya purchasi*, has been employed for over a century. Vedalia beetles are specialists, multivoltine, long-lived and highly efficient in obtaining prey [22]. Importantly, these are all ecological/phenotypic traits that can be characterized readily using genomic approaches [110,111]. Thus, experimental evolution and/or simulation studies based on existing genomic diversity of populations of the vedalia beetle (and other biological control organisms) could elucidate the effects of standing genomic variation on adaptability to novel environments. Efforts to quantify such variation in insect predators, including transcriptome and mitochondrial genome sequencing of *C. septempunctata* [112,113] and whole genome sequencing of *H. axyridis* (Havens et al., http://f1000research.com/posters/1096169), are underway. Additionally, a growing literature on landscape genomics methods (summarized in [114,115]) highlights incorporating models of the distribution of populations in integrative studies of ecological and genomic variation [116]. Ultimately, new methods and software for jointly estimating demography and ecological parameters using genomic and geographical data should prove indispensable in studying the establishment of biological control organisms in novel environments.
4.2. Suggested Pipeline For Including Genomics Into Biological Control Programs

Rendering biological control more predictable, thus increasing the estimated 10 percent of reported attempts being successful, has been a long-term goal of applied ecologists and entomologists Gurr & Wratten, 1999 and Gurr et al., 2012 [2,13] argue that a majority of this failure rate concerns disregard for habitat requirements of the biological control organisms. They suggest that microhabitat manipulation (host ranges, prey/food availability, microclimates, etc.) ought to improve the chances of success. Although arguably true in several cases [117], genetic drift, natural selection and non-random mating surely play important and yet often undetermined roles as well [12,118,119]. However, predicting the evolutionary responses of organisms utilized in biological control is no easy task, as the number of contributing factors is formidable. Here we suggest four major population genetic processes—population size change, selection, gene flow and inbreeding—that, when quantified, can proffer important evidence of short- and long-term evolutionary trajectories of introduced organisms and their target species. Plummeting sequencing and genotyping costs and accelerated development of statistical methods and population genomics pipelines to estimate evolutionary parameters under a variety of demographic models, render these crucial insights more accessible. Thus, we propose a nine-step paradigm based on evolutionary and ecological principles—a ‘pipeline’ for applied ecologists and entomologists to enhance the likelihood of successful biological control programs.

1. Define biological questions about the system and build a hypothesized quantitative model of evolution based on mode of biological control. Is there a historical record of introductions in other regions, trophic-level interactions and ecological success parameters (described in Reference [13], including census size estimation and range expansion with host? For example, H. axyridis has successfully established populations across the world owing to importation for biological control and invasiveness. Due to its known historical record of introduction, Lombaert et al., 2010 [20] propose and test a model of hybridization of inbred Eastern and Western clusters of the species that putatively yielded the invasive Eastern North American population.

2. Develop a sampling plan. Numerous studies [120,121] describe the issue of sample sizes, determined as (a) the number of individuals sampled per locale, (b) the number of sampling locales, (c) and the number and type of genomic loci analyzed. In short, although large sample sizes are preferable for estimating genomic diversity and differentiation, coalescent modeling and estimation of evolutionary history can work well with smaller sample sizes and greater number of genomic loci. Using replicated random samples of 3000 SNPs (Single Nucleotide Polymorphisms) from a large 2bRAD dataset from populations of the biological control organism H. axyridis, Li et al., 2020 [122] determined that a minimum of 6 individuals per population are sufficient to accurately estimate within- and between-population genomic diversity and differentiation. The ideal sampling plan should also be informed by the sequencing platform or protocol used for genotyping-by-sequencing, which is optimized to run up to 96 uniquely barcoded individuals to obtain thousands of informative sites.

3. Conduct genotyping/sequencing. Strategies for obtaining molecular sequence or genotype information are contingent primarily on previously available genomic information from the species of interest. For example, many arthropod genomes are currently available (476 as of May 2020), with more in the works (see Arthropod Genomic Consortium, http://i5k.github.io/arthropod_genomes_at_ncbi) [123]. Alternatively, dense reduced representation library-based sequencing/genotyping [124] via technologies like RADseq [125] and PoolSeq [126] offer opportunities for demographic inference using SNPs in species with little prior genomic information. Meanwhile, repeat-based markers such as microsatellites continue to provide useful genetic insights into biological control organisms [20,21,98,127].

4. Undertake preliminary bioinformatics steps involved in sequence/genotype clean-up, assembly, alignment and variant calling. Pipelines and tools have been developed to ease
processing genomic/genotypic/sequencing data, including GATK [128], vcf tools [129], SAMTools [130], BAMTools [131] and STACKS [132]. Resources for preliminary bioinformatics analyses are summarized under contributions of the Galaxy Project (www.galaxyproject.org) [133,134].

5. Perform exploratory analyses. Calculate Method of Moments estimates of summary statistics, including heterozygosity, polymorphism, diversity indices, differentiation, allelic richness and inbreeding coefficients. Tools that bundle methods to estimate most basic summary statistics from genomic data include STACKS [132], VCFTools [129], PopGenome [135] and adegenet/pegas [136,137] packages in R (Table 3).

6. Perform secondary analyses. Build data-sets (from whole genomic, reduced representation or genotypic data) that satisfy assumptions of the model or method of choice. Each method listed in Table 1 has its own set of caveats, assumptions and models, more details about which have been summarized in Reference [138].

7. Simulate/estimate parameters under the model. The choice of programs for estimating demographic parameters depends on the type of genomic data (Table 1). Genotypic data (e.g., SNPs) are amenable for use in frequency-based statistics to infer demography and processes of divergent evolution. For instance, using SNP loci to compute divergence statistics (Fst—[139] and other variants—[140,141], D statistic—ABBA-BABA tests—see References [60,142] can reveal migratory history between populations. Similarly, allele frequencies computed from individual loci can be used in likelihood and Bayesian methods to estimate population genetic structure and admixture, which is the basis of the widely cited program, STRUCTURE [41]. With ongoing improvements in sequencing technologies that offer high coverage and long reads, genotyping-by-sequencing technologies likely will be the go-to in terms of generating and analyzing large-scale population genomic data for biological control where no extensive whole genomic resources are available currently.

8. Model selection. Demographic models often oversimplify the irrefutably complex reality of how populations evolve. However, statistics allow us to rigorously identify a model that explains the data better. Depending on the statistical methods applied, commonly utilized model-selection paradigms include likelihood ratio tests [54] and Akaike/Bayesian Information Criteria [143].

9. Interpret estimated parameters under the “best” model, reconciling assumptions and biology of the system. The final step involves using a statistically informed explanation of the biological processes affecting populations of introduced biological control organisms and discussing the caveats of using model-based population genomics.

Table 3. List of commonly used tools/pipelines for preliminary analyses (data compilation, assembly, filtering, quality control, formatting) of population genomic data.

| Software       | Citation              | Type of Data                  | Purpose                                                        |
|----------------|-----------------------|-------------------------------|----------------------------------------------------------------|
| VCFTOOLS       | Danecek et al., 2011 [129] | Genomic, SNP               | Variant calling, summary statistics, data filtering, file manipulation |
| SAMTOOLS       | Li et al., 2009 [130]  | Genomic, multiple sequence alignment | Data filtering, cleanup, multiple sequence alignment, file manipulation |
| BAMTOOLS       | Barnett et al., 2011 [131] | Genomic, multiple sequence alignment | Data filtering, cleanup, multiple sequence alignment, file manipulation |
| GATK           | McKenna et al., 2010 [128] | Genomic, SNP               | Variant calling, summary statistics, data filtering             |
| GALAXY PROJECT | Blankenberg et al., 2010 [134] | All                        | Suite of pipelines for numerous bioinformatics analyses of genomic data |
| JVARKIT        | Lindenbaum 2015 [144]  | Genomic, SNP               | Suite of tools for data filtering, file manipulation, cleanup   |
| SNP-SITES      | Page et al., 2016 [145] | Genomic, SNP               | Variant calling                                               |
| BIOCONDUCTOR   | Gentleman et al., 2004 [146] | All                        | Suite of pipelines for numerous bioinformatics analyses of genomic data |
| ADEGENET/PEGAS | Jombart 2008 [136], Paradis 2010 [137] | Genomic, SNP               | Suite of tools for data filtering, file manipulation, cleanup |
| POPGENOME      | Pfeifer et al., 2014 [135] | Genomic, multiple sequence alignment | Suite of tools for data filtering, file manipulation, cleanup |


5. Conclusions

Beginning with the development of biological control as a major tactic for pest management during the 20th century, an appreciation that biological control was not only applied ecology but also had a foundation in genetics and evolution, was gained. Still, for most of the 1900s, the major emphasis of the discipline remained on ecological principles, with notable exceptions [152–154]. During the past 25 years, as molecular tools have been applied to address evolutionary questions in biological control, we have gained a deeper appreciation of transgenerational processes. Emerging topics examined in relation to biological control include manipulating genetic variation in biological control organisms [155], using molecular tools in importation biological control [156], revealing microevolution [17] and examining evolutionary concepts in importation biological control [157,158]. In this spirit, Evolutionary Applications dedicated an issue to focus on evolution and biological control [159]. Within this scholarly work, an appreciation of the influence of new cutting-edge tools on the discipline was recognized. For example, Roderick et al. [159] identified next-generation sequencing, computational modeling and bioinformatics as approaches that would enhance our understanding of evolution in biological control. In our review, we specifically focus on harnessing the power of population genomics, including next-generation sequencing and demographic modeling, to provide a more predictive basis and evolutionary understanding for biological control. With the rapid development and application of sophisticated molecular and computational tools and approaches, we show how new perspectives and insights can be gained on long-standing questions related to the genetic bases and evolutionary outcomes of human manipulation of biological control organisms for the management of pest species.

Funding: This work was funded by USDA grant #2017-06423 to Drs. George Vourlitis and Arun Sethuraman. AS was also supported by NSF ABI Development grant #1564659.

Acknowledgments: We thank members of the Sethuraman Lab for their help with proofreading this review.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gutierrez, A.; Caltagirone, L.; Meikle, W. Evaluation of results: Economics of biological control. In *Handbook of Biological Control*; Elsevier: Amsterdam, The Netherlands, 1999; pp. 243–252.
2. Gurr, G.; Wratten, S. FORUM 'Integrated biological control': A proposal for enhancing success in biological control. *Int. J. Pest Manag.* 1999, 45, 81–84.
3. Gurr, G.; Wratten, S.; Barbosa, P. Success in conservation biological control of arthropods. In *Biological control: Measures of Success*; Springer: Berlin/Heidelberg, Germany, 2000; pp. 105–132.
4. Fisher, T.; Bellows, T.S.; Caltagirone, L.; Dahlsten, D.; Huffaker, C.B.; Gordh, G. *Handbook of Biological Control: Principles and Applications of Biological Control*; Elsevier: Amsterdam, The Netherlands, 1999.
5. Pickett, C.; Pitcairn, M. Classical biological control of ash whitefly: Factors contributing to its success in California. *BioControl* 1999, 44, 143–158.
6. Van Driesche, R.G.; Bellows, T.S. Biology of arthropod parasitoids and predators. In *Biological Control*; Springer: Berlin/Heidelberg, Germany, 1996; pp. 309–336.
7. Neuenschwander, P. Biological control of the cassava mealybug in Africa: A review. *Biol. Control* 2001, 21, 214–229.
8. Haynes, D.; Gage, S. The cereal leaf beetle in North America. *Annu. Rev. Entomol.* **1981**, *26*, 259–287.
9. Hajek, A.E.; Eilenberg, J. *Natural Enemies: An Introduction to Biological Control*; Cambridge University Press: Cambridge, UK, 2018.
10. Wainberg, E.; Scott, J.K.; Quimby, P.C. Evaluating Indirect Ecological Effects of Biological Control; CABI: Wallingford, UK, 2001.
11. Follett, P.A.; Duan, J.J. *Nontarget Effects of Biological Control*; Springer: Berlin/Heidelberg, Germany, 2012.
12. Ehler, L.E.; Sforza, R.; Mateille, T. *Genetics, Evolution, and Biological Control*; CABI: Wallingford, UK, 2003.
13. Gurr, G.M.; Wratten, S.D.; Snyder, W.E. Biodiversity and Insect Pests: Key Issues for Sustainable Management; John Wiley & Sons: Hoboken, NJ, USA, 2012.
14. Guillemaud, T.; Ciosi, M.; Lombaert, E.; Estoup, A. Biological invasions in agricultural settings: Insights from evolutionary biology and population genetics. *Comptes Rendus Biol.* **2011**, *334*, 237–246.
15. Gassmann, A.J.; Onstad, D.W.; Pittendrigh, B.R. Evolutionary analysis of herbivorous insects in natural and agricultural environments. *Pest. Manag. Sci. Former. Pestic. Sci.* **2009**, *65*, 1174–1181.
16. Rius, M.; Bourne, S.; Hornsby, H.G.; Chapman, M.A. Applications of next-generation sequencing to the study of biological invasions. *Curr. Zool.* **2015**, *61*, 488–504.
17. Hufbauer, R.A.; Roderick, G.K. Microevolution in biological control: Mechanisms, patterns, and processes. *Biol. Control* **2005**, *35*, 227–239.
18. Clausen, C. Introduced parasites and predators of arthropod pests and weeds: A world review. *Curculionidae. US Dep. Agric. Agric. Handb.* 480, 1978, pp. 259–276
19. Kajita, Y.; O'Neill, E.M.; Zheng, Y.; Obrycki, J.J.; Weisrock, D.W. A population genetic signature of human releases in an invasive ladybeetle. *Mol. Ecol.* **2012**, *21*, 5473–5483.
20. Lombaert, E.; Guillemaud, T.; Cornuet, J.-M.; Malausa, T.; Facon, B.; Estoup, A. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. *PLoS ONE* **2010**, *5*, e9743.
21. Lombaert, E.; Estoup, A.; Facon, B.; Joubard, B.; Grégoire, J.-C.; Jannin, A.; Blin, A.; Guillemaud, T. Rapid increase in dispersal during range expansion in the invasive ladybird Harmonia axyridis. *J. Evol. Biol.* **2014**, *27*, 508–517.
22. Caltagirone, L.; Doutt, R. The history of the vedalia beetle importation to California and its impact on the development of biological control. *Annu. Rev. Entomol.* **1989**, *34*, 1–16.
23. Slatkin, M.; Excoffier, L. Serial founder effects during range expansion: A spatial analog of genetic drift. *Genetics* **2012**, *191*, 171–181.
24. Talamas, E.J.; Herlihy, M.V.; Dieckhoff, C.; Hoelmer, K.A.; Buffington, M.; Bon, M.-C.; Weber, D.C. *Trissolcus japonicus* (Ashmead)(Hymenoptera, Scelionidae) emerges in North America. *J. Hymenopt. Res.* **2015**, *43*, 119.
25. Estoup, A.; Guillemaud, T. Reconstructing routes of invasion using genetic data: Why, how and so what? *Mol. Ecol.* **2010**, *19*, 4113–4130.
26. Calfee, E.; Agra, M.N.; Palacio, M.A.; Ramírez, S.R.; Coop, G. Selection and hybridization shaped the Africanized honey bee invasion of the Americas. *BioRxiv* 2020, doi:10.1101/2020.03.17.994632.
27. Van Lenteren, J. Success in biological control of arthropods by augmentation of natural enemies. In *Biological Control: Measures of Success*; Springer: Berlin/Heidelberg, Germany, 2000; pp. 77–103.
28. Elzen, G.W.; King, E.G. Periodic release and manipulation of natural enemies. In *Handbook of Biological Control*; Elsevier: Amsterdam, The Netherlands, 1999; pp. 253–270.
29. Van Lenteren, J.C. The state of commercial augmentative biological control: Plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **2012**, *57*, 1–20.
30. Collier, T.; Van Steenwyk, R. A critical evaluation of augmentative biological control. *Biol. Control* **2004**, *31*, 245–256.
31. Brewer, M.J.; Nelson, D.J.; Ahern, R.G.; Donahue, J.D.; Prokrym, D.R. Recovery and range expansion of parasitoids (Hymenoptera: Apfeliniidae and Braconidae) released for biological control of *Diuraphis noxia* (Homoptera: Aphididae) in Wyoming. *Environ. Entomol.* **2001**, *30*, 578–588.
32. Louda, S.M.; Kendall, D.; Connor, J.; Simberloff, D. Ecological effects of an insect introduced for the biological control of weeds. *Science* **1997**, *277*, 1088–1090.
33. Louda, S.M.; Pemberton, R.; Johnson, M.; Follett, P. Nontarget effects—The Achilles’ heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annu. Rev. Entomol.* **2003**, *48*, 365–396.
34. Simberloff, D.; Stiling, P. How risky is biological control? *Ecology* **1996**, *77*, 1965–1974.
35. Wright, S. Isolation by distance. Genetics 1943, 28, 114.
36. Rousset, F. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 1997, 145, 1219–1228.
37. Aguillon, S.M.; Fitzpatrick, J.W.; Bowman, R.; Schoech, S.J.; Clark, A.G.; Coop, G.; Chen, N. Deconstructing isolation-by-distance: The genomic consequences of limited dispersal. PLoS Genet. 2017, 13, e1006911, doi:10.1371/journal.pgen.1006911.
38. Williamson, M.H.; Fitter, A. The characters of successful invaders. Biol. Conserv. 1996, 78, 163–170.
39. Maruyama, T.; Fuerst, P.A. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 1985, 111, 675–689.
40. Cornuet, J.M.; Luikart, G. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 1996, 144, 2001–2014.
41. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. Genetics 2000, 155, 945–959.
42. Wu, B.; Liu, N.; Zhao, H. PSMIX: An R package for population structure inference via maximum likelihood method. BMC Bioinform. 2006, 7, 317.
43. Alexander, D.H.; Novembre, J.; Lange, K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 2009, 19, 1655–1664.
44. Tang, H.; Peng, J.; Wang, P.; Risch, N.J. Estimation of individual admixture: Analytical and study design considerations. Genet. Epidemiol. Off. Publ. Int. Genet. Epidemiol. Soc. 2005, 28, 289–301.
45. Price, A.L.; Patterson, N.J.; Plenge, R.M.; Weinblatt, M.E.; Shadick, N.A.; Reich, D. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 2006, 38, 904–909.
46. Hey, J.; Nielsen, R. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of Drosophila pseudoobscura and D. persimilis. Genetics 2004, 167, 747–760.
47. Hey, J.; Nielsen, R. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proc. Natl. Acad. Sci. USA 2007, 104, 2785–2790.
48. Hey, J. Isolation with migration models for more than two populations. Mol. Biol. Evol. 2010, 27, 905–920.
49. Sethuraman, A.; Hey, J. IM a2p–parallel MCMC and inference of ancient demography under the Isolation with migration (IM) model. Mol. Ecol. Resour. 2016, 16, 206–215.
50. Beerli, P.; Felsenstein, J. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 2001, 98, 4563–4568.
51. Beerli, P.; Felsenstein, J. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 1999, 152, 763–773.
52. Beerli, P. MIGRATE-N: Estimation of population sizes and gene flow using the coalescent. 2008. Available online: popgen.sc.fsu.edu/Migrate/Download.html (accessed on: 1 June 2020).
53. Wilson, G.A.; Rannala, B. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 2003, 163, 1177–1191.
54. Nielsen, R.; Wakeley, J. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. Genetics 2001, 158, 885–896.
55. Kuhner, M.K. LAMARC 2.0: Maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 2006, 22, 768–770.
56. Cornuet, J.-M.; Ravaigué, V.; Estoup, A. Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC Bioinform. 2010, 11, 401.
57. Beaumont, M.A. Estimation of population growth or decline in genetically monitored populations. Genetics 2003, 164, 1139–1160.
58. Chen, C.; Forbes, F.; François, O. fastruct: Model-based clustering made faster. Mol. Ecol. Notes 2006, 6, 980–983.
59. Corander, J.; Marttinen, P. Bayesian identification of admixture events using multilocus molecular markers. Mol. Ecol. 2006, 15, 2833–2843.
60. Patterson, N.; Moorjani, P.; Luo, Y.; Mallick, S.; Rohland, N.; Zhan, Y.; Genschoreck, T.; Webster, T.; Reich, D. Ancient admixture in human history. Genetics 2012, 192, 1065–1093.
61. Pickrell, J.; Pritchard, J. Inference of population splits and mixtures from genome-wide allele frequency data. Nat. Preced. 2012, 8, doi:10.1371/journal.pgen.1002967.
62. Kuhner, M.K.; Yamato, J.; Felsenstein, J. Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics* 1998, 149, 429–434.
63. Raj, A.; Stephens, M.; Pritchard, J.K. fastSTRUCTURE: Variational inference of population structure in large SNP data sets. *Genetics* 2014, 197, 573–589.
64. Gronau, I.; Hubisz, M.J.; Gulko, B.; Danko, C.G.; Siepel, A. Bayesian inference of ancient human demography from individual genome sequences. *Nat. Genet.* 2011, 43, 1031.
65. Li, H.; Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* 2010, 26, 589–595.
66. Excoffier, L.; Dupanloup, I.; Huerta-Sánchez, E.; Sousa, V.C.; Foll, M. Robust demographic inference from genomic and SNP data. *PLoS Genet.* 2013, 9, e1003905, doi:10.1371/journal.pgen.1003905.
67. Gutenkunst, R.; Hernandez, R.; Williamson, S.; Bustamante, C. Diffusion approximations for demographic inference: DaDi. *Nat. Preced.* 2010, doi:10.1038/npre.2010.4594.1.
68. Excoffier, C.L.D.W.L. Bayesian computation and model selection in population genetics. *arXiv Prepr.* 2009, arXiv:0901.2231.
69. Hulsenbeck, J.P.; Andolfatto, P.; Hulsenbeck, E.T. Structurama: Bayesian inference of population structure. *Ecol. Bioinform.* 2011, 7, EBO-S6761.
70. Sheehan, S.; Harris, K.; Song, Y.S. Estimating variable effective population sizes from multiple genomes: A sequentially Markov conditional sampling distribution approach. *Genetics* 2013, 194, 647–662.
71. Pavlidis, P.; Živković, D.; Stanatakis, A.; Alachiotis, N. SweeD: Likelihood-based detection of selective sweeps in thousands of genomes. *Mol. Biol. Evol.* 2015, 30, 2224–2234.
72. DeGiorgio, M.; Huber, C.D.; Hubisz, M.J.; Hellmann, I.; Nielsen, R. SweepFinder2: Increased sensitivity, robustness and flexibility. *Bioinformatics* 2016, 32, 1895–1897.
73. Wang, J.; Whitlock, M.C. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 2003, 163, 429–446.
74. Do, C.; Waples, R.S.; Peel, D.; Macbeth, G.; Tillett, B.J.; Ovenden, J.R. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Mol. Ecol. Resour.* 2014, 14, 209–214.
75. Fauvergue, X.; Vercken, E.; Malaua, T.; Hufbauer, R.A. The biology of small, introduced populations, with special reference to biological control. *Evol. Appl.* 2012, 5, 424–443.
76. Franks, S.J.; Pratt, P.D.; Tsutsui, N.D. The genetic consequences of a demographic bottleneck in an introduced biological control insect. *Conserv. Genet.* 2011, 12, 201–211.
77. Estoup, A.; Wilson, I.J.; Sullivan, C.; Cornuet, J.-M.; Moritz, C. Inferring population history from microsatellite and enzyme data in serially introduced cane toads, Bufo marinus. *Genetics* 2001, 159, 1671–1687.
78. Fowler, S.V.; Peterson, P.; Barrett, D.P.; Forgie, S.; Gleeson, D.M.; Harman, H.; Houliston, G.J.; Smith, L. Investigating the poor performance of heather beetle, *Lochmaea suturalis* (Thompson) (Coleoptera: Chrysomelidae), as a weed biocontrol agent in New Zealand: Has genetic bottlenecks resulting in small body size and poor winter survival? *BioL Control.* 2015, 87, 32–38.
79. Roy, H.; Wajnberg, E. From biological control to invasion: The ladybird *Harmonia axyridis* as a model species. *BioControl* 2008, 53, 1–4.
80. Davidson, A.M.; Jennions, M.; Nicostra, A.B. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.* 2011, 14, 419–431.
81. Fischer, M.J.; Havill, N.P.; Brewster, C.C.; Davis, G.A.; Salom, S.M.; Kok, L.T. Field assessment of hybridization between *Laricobius nigrinus* and *L. rubidus*, predators of Adelgidae. *BioL Control.* 2015, 82, 1–6.
82. Tayeh, A.; Hufbauer, R.A.; Estoup, A.; Ravné, V.; Frachon, L.; Facon, B. Biological invasion and biological control select for different life histories. *Nat. Commun.* 2015, 6, 1–5.
83. Nielsen, R. Molecular signatures of natural selection. *Annu. Rev. Genet.* 2005, 39, 197–218.
84. Phillips, P.C.; Arnold, S.J. Visualizing multivariate selection. *Evolution* 1989, 43, 1209–1222.
85. Phillips, C.; Baird, D.; Ilie, I.; McNeill, M.; Prowfitt, J.; Goldson, S.; Kean, J. East meets west: Adaptive evolution of an insect introduced for biological control. *J. Appl. Ecol.* 2008, 45, 948–956.
86. Dlugosch, K.M.; Parker, I.M. Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 2008, 17, 431–449.
87. Kolbe, J.J.; Glor, R.E.; Schettino, L.R.; Lara, A.C.; Larson, A.; Losos, J.B. Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **2004**, *431*, 177–181.

88. Turner, K.G.; Hufbauer, R.A.; Rieseberg, L.H. Rapid evolution of an invasive weed. *New Phytol.* **2014**, *202*, 309–321.

89. Goldson, S.; Wratten, S.; Ferguson, C.; Gerard, P.; Barratt, B.; Hardwick, S.; McNeill, M.; Phillips, C.; Popay, A.; Tylianakis, J.; et al. If and when successful classical biological control fails. *Biol. Control* **2014**, *72*, 76–79.

90. Biondi, A.; Desneux, N.; Siscaro, G.; Zappalà, L. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* **2012**, *87*, 803–812.

91. Heimpel, G.E.; Asplen, M.K. A ‘Goldilocks’ hypothesis for dispersal of biological control agents. *BioControl* **2011**, *56*, 441–450.

92. Welch, J.J.; Jiggins, C.D. Standing and flowing: The complex origins of adaptive variation. *Mol. Ecol.* **2014**, *23*, 3935–3937.

93. Cruickshank, T.E.; Hahn, M.W. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Mol. Ecol.* **2014**, *23*, 3133–3157.

94. Hufbauer, R.A.; Szucs, M.; Kasyon, E.; Youngberg, C.; Koontz, M.J.; Richards, C.; Tuff, T.; Melbourne, B.A. Three types of rescue can avert extinction in a changing environment. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 10557–10562.

95. Hufbauer, R.; Rutschmann, A.; Serrate, B.; Vermeil de Conchard, H.; Facon, B. Role of propagule pressure in colonization success: Disentangling the relative importance of demographic, genetic and habitat effects. *J. Ecol. Biol.* **2013**, *26*, 1691–1699.

96. Facon, B.; Hufbauer, R.A.; Tayeh, A.; Loiseau, A.; Lombaert, E.; Vitalis, R.; Guillemaud, T.; Lundgren, J.G.; Estoup, A. Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Curr. Biol.* **2011**, *21*, 424–427.

97. Obrycki, J.J.; Krafsur, E.S.; Bogran, C.E.; Gomez, L.E.; Cave, R.E. Comparative studies of three populations of the lady beetle predator *Hipodamia convergens* (*Coleoptera: Coccinellidae*). *Fla. Entomol.* **2001**, *84*, 55–62.

98. Sethuraman, A.; Janzen, F.J.; Obrycki, J. Population genetics of the predatory lady beetle *Hipodamia convergens*. *BioControl* **2015**, *84*, 1–10.

99. Turgeon, J.; Tayeh, A.; Facon, B.; Lombaert, E.; De Clercq, P.; Berkvens, N.; Lundgren, J.; Estoup, A. Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. *J. Evol. Biol.* **2011**, *24*, 1044–1052.

100. Szucs, M.; Schaffner, U.; Price, W.J.; Schwarzländer, M. Post-introduction evolution in the biological control agent *Longitarsus jacobaeae* (*Coleoptera: Chrysomelidae*). *Evol. Appl.* **2012**, *5*, 858–868.

101. Havill, N.P.; Davis, G.; Mausel, D.L.; Klein, J.; McDonald, R.; Jones, C.; Fischer, M.; Salom, S.; Caccione, A. Hybridization between a native and introduced predator of *Adelgidae*: An unintended result of classical biological control. *BioControl* **2012**, *63*, 359–369.

102. Hedrick, P.W. Adaptive introgression in animals: Examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* **2013**, *22*, 4606–4618.

103. Arnold, M.L.; Martin, N.H. Adaptation by introgression. *J. Biol.* **2009**, *8*, 82.

104. Lenormand, T. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **2002**, *17*, 183–189.

105. Rymer, J.M.; Simberloff, D. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **1996**, *27*, 83–109.

106. Blackburn, T.M.; Lockwood, J.L.; Cassey, P. The influence of numbers on invasion success. *Mol. Ecol.* **2015**, *24*, 1942–1953.

107. Grenier, C.; Summerhayes, B.; Cartmill, R.; Martinez, T.; Saisho, R.; Rothenberg, A.; Scott, J.; Obrycki, J.; Sethuraman, A. Lack of phenotypic variation in larval utilization of pea aphids in populations of the ladybeetle *Hipodamia convergens*. *bioRxiv* 2019, 740506.

108. Stiling, P.; Cornelissen, T. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Bio. Control* **2005**, *34*, 236–246.

109. Gilbert, K.J.; Whitlock, M.C. Evaluating methods for estimating local effective population size with and without migration. *Evolution* **2015**, *69*, 2154–2166.

110. Tribolium Genome Sequencing Consortium. The genome of the model beetle and pest *Tribolium castaneum*. *Nature* **2008**, *452*, 949–955.
111. Keeling, C.I.; Yuen, M.M.; Liao, N.Y.; Docking, T.R.; Chan, S.K.; Taylor, G.A.; Palmquist, D.L.; Jackman, S.D.; Nguyen, A.; Li, M.; et al. Draft genome of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major forest pest. *Genome Biol.* 2013, 14, R27.

112. Qi, X.; Zhang, L.; Han, Y.; Ren, X.; Huang, J.; Chen, H. De novo transcriptome sequencing and analysis of *Coccinella septempunctata* L. in non-diapause, diapause and diapause-terminated states to identify diapause-associated genes. *BMC Genom.* 2015, 16, 1086.

113. Kim, M.J.; Wan, X.; Kim, I. Complete mitochondrial genome of the seven-spotted lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Mitochondrial DNA* 2012, 23, 179–181.

114. Sork, V.; Aitken, S.; Dyer, R.; Eckert, A.; Legendre, P.; Neale, D. Putting the landscape into the genomics of trees: Approaches for understanding local adaptation and population responses to changing climate. *Tree Genet. Genomes* 2013, 9, 901–911.

115. Joost, S.; Vuilleumier, S.; Jensen, J.D.; Schoville, S.; Leempoel, K.; Stucki, S.; Widmer, I.; Melodelima, C.; Rolland, J.; Manel, S. Uncovering the genetic basis of adaptive change: On the intersection of landscape genomics and theoretical population genetics. *Mol. Ecol.* 2013, 22, 3659–3665.

116. Schwartz, M.K.; McKelvey, K.S.; Cushman, S.A.; Luikart, G. Landscape genomics: A brief perspective. In *Spatial Complexity, Informatics, and Wildlife Conservation*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 165–174.

117. Gurr, G.; Wrenn, S. *Biological control: Measures of Success*; Springer: Berlin/Heidelberg, Germany, 2000;

118. Wajnberg, E. Measuring genetic variation in natural enemies used for biological control: Why and how. *Genet. Evol. Biol. Control* 2004, 19–37. doi:10.1079/9780851997353.0019.

119. Roderick, G. Tracing the origin of pests and natural enemies: Genetic and statistical approaches. *Genet. Evol. Biol. Control* 2004, 97–112. doi:10.1079/9780851997353.0097.

120. Nielsen, R.; Beaumont, M.A. Statistical inferences in phylogeography. *Mol. Ecol.* 2009, 18, 1034–1047.

121. Felsenstein, J. Accuracy of coalescent likelihood estimates: Do we need more sites, more sequences, or more loci? *Mol. Biol. Evol.* 2006, 23, 691–700.

122. Li, H.; Qu, W.; Obrycki, J.J.; Meng, L.; Zhou, X.; Chu, D.; Li, B. Optimizing Sample Size for Population Genomic Study in a Global Invasive Lady Beetle, *Harmonia axyridis*. *Insects* 2020, 11, 290.

123. Thomas, G.W.; Dohmen, E.; Hughes, D.S.; Murali, S.C.; Poelchau, M.; Glastad, K.; Arstead, C.A.; Ayoub, N.A.; Batterham, P.; Bellair, M.; et al. Gene content evolution in the arthropods. *Genome Biol.* 2020, 21, 1–14.

124. Davey, J.W.; Hohenlohe, P.A.; Etter, P.D.; Boone, J.Q.; Catchen, J.M.; Blaxter, M.L. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* 2011, 12, 499–510.

125. Andrews, K.R.; Good, J.M.; Miller, M.R.; Luikart, G.; Hohenlohe, P.A. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat. Rev. Genet.* 2016, 17, 81.

126. Schlötterer, C.; Tebeler, R.; Kofler, R.; Nolte, V. Sequencing pools of individuals—Mining genome-wide polymorphism data without big funding. *Nat. Rev. Genet.* 2014, 15, 749–763.

127. Sethuraman, A.; Janzen, F.J.; Rubio, M.A.; Vasquez, Y.; Obrycki, J.J. Demographic histories of three predatory lady beetles reveal complex patterns of diversity and population size change in the United States. *Insect Sci.* 2018, 25, 1065–1079.

128. McKenna, A.; Hanna, M.; Banks, E.; Sivachenko, A.; Cibulskis, K.; Kernytsky, A.; Garmella, K.; Altshuler, D.; Gabriel, S.; Daly, M.; et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010, 20, 1297–1303.

129. Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; DePristo, M.A.; Handsaker, R.E.; Lunter, G.; Marth, G.T.; Sherry, S.T.; et al. The variant call format and VCFtools. *Bioinformatics* 2011, 27, 2156–2158.

130. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009, 25, 2078–2079.

131. Barnett, D.W.; Garrison, E.K.; Quinlan, A.R.; Strömberg, M.P.; Marth, G.T. BamTools: A C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics* 2011, 27, 1691–1692.

132. Catchen, J.M.; Amores, A.; Hohenlohe, P.; Cresko, W.; Postlethwait, J.H. Stacks: Building and genotyping loci de novo from short-read sequences. *G3 GenesGenomesGenet.* 2011, 1, 171–182.

133. Goecks, J.; Nekrutenko, A.; Taylor, J.; Team, G.; others Galaxy: A comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* 2010, 11, R86.

134. Blankenberg, D.; Kuster, G.V.; Coraor, N.; Ananda, G.; Lazarus, R.; Mangan, M.; Nekrutenko, A.; Taylor, J. Galaxy: A web-based genome analysis tool for experimentalists. *Curr. Protoc. Mol. Biol.* 2010, 89, 19–10.
135. Pfeifer, B.; Wittelsbürger, U.; Ramos-Onsins, S.E.; Lercher, M.J. PopGenome: An efficient Swiss army knife for population genomic analyses in R. *Mol. Biol. Evol.* 2014, 31, 1929–1936.

136. Jombart, T. adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics* 2008, 24, 1403–1405.

137. Paradis, E. pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics* 2010, 26, 419–420.

138. Schraiber, J.G.; Akey, J.M. Methods and models for unravelling human evolutionary history. *Nat. Rev. Genet.* 2015, 16, 727–740.

139. Wright, S. The genetical structure of populations. *Ann. Eugen.* 1949, 15, 323–354.

140. Nei, M.; Chesser, R.K. Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.* 1983, 47, 253–259.

141. Weir, B.S.; Cockerham, C.C. Estimating F-statistics for the analysis of population structure. *Evolution* 1984, 38, 1358–1370.

142. Durand, E.Y.; Patterson, N.; Reich, D.; Slatkin, M. Testing for ancient admixture between closely related populations. *Mol. Biol. Evol.* 2011, 28, 2239–2252.

143. Johnson, J.B.; Omland, K.S. Model selection in ecology and evolution. *Trends Ecol. Evol.* 2004, 19, 101–108.

144. Lindenbaum, P. Jvarkit: Java utilities for bioinformatics. 2015. Available online: https://figshare.com/articles/Jvarkit_java_based_utilities_for_Bioinformatics/1425030 (accessed on: 1 June 2020).

145. Page, A.J.; Taylor, B.; Delaney, A.J.; Soares, J.; Seemann, T.; Keane, J.A.; Harris, S.R. SNP-sites: Rapid efficient extraction of SNP’s from multi-FASTA alignments. *Microb. Genom.* 2016, 2, e000056, doi:10.1099/mgen.0.000056.

146. Gentleman, R.C.; Carey, V.J.; Bates, D.M.; Bolstad, B.; Dettling, M.; Dudoit, S.; Ellis, B.; Gautier, L.; Ge, Y.; Gentry, J.; et al. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol.* 2004, 5, R80.

147. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 2013, 30, 2725–2729.

148. Rousset, F. Inbreeding and relatedness coefficients: What do they measure? *Heredity* 2002, 88, 371–380.

149. Excoffier, L.; Lischer, H.E. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* 2010, 10, 564–567.

150. Librado, P.; Rozas, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009, 25, 1451–1452.

151. Quinlan, A.R. BEDTools: The Swiss-army tool for genome feature analysis. *Curr. Protoc. Bioinform.* 2014, 47, 11–12.

152. Whitten, M.; Hoy, M.A. Genetic improvement and other genetic considerations for improving the efficacy and success rate of biological control. In *Handbook of Biological Control*; Elsevier: Amsterdam, The Netherlands, 1999; pp. 271–296.

153. Mackauer, M. Genetic problems in the production of biological control agents. *Annu. Rev. Entomol.* 1976, 21, 369–385.

154. Messenger, P.; Wilson, F.; Whitten, M. Variation, fitness, and adaptability of natural enemies. In *Theory Pract. Biol. Control* 1976, Elsevier: Amsterdam, The Netherlands, pp 209–231.

155. Hopper, K.; Roush, R.T.; Powell, W. Management of genetics of biological-control introductions. *Annu. Rev. Entomol.* 1993, 38, 27–51.

156. Unruh, T.; Woolley, J. Molecular Methods in Classical Biological Control. In *Handbook of Biological Control*; 1999, Elsevier: Amsterdam, The Netherlands, pp. 57–85.

157. Roderick, G.K.; Navajas, M. Genes in new environments: Genetics and evolution in biological control. *Nat. Rev. Genet.* 2003, 4, 889–899.

158. Roderick, G.; Navajas, M. The primacy of evolution in biological control. In Proceedings of the XII International Symposium on Biological Control of Weeds: La Grande Motte, France; 22–27 April 2007; pp. 22–27.

159. Roderick, G.K.; Hufbauer, R.; Navajas, M. Evolution and biological control. *Evol. Appl.* 2012, 5, 419.
