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RESEARCH ARTICLE

BugBook: Genetics of insects as food and feed

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Abstract

Just like traditional livestock, farmed insects harbour genetic variation and can be selectively bred to optimise traits of interest. This BugBook article presents a comprehensive overview of how genetics can contribute to improving insect production for food and feed. Molecular genetics and genomics approaches for generating the data essential for understanding species biology are presented, as well as their implementation in dedicated selective breeding programmes, and options for health and quality control of mass rearing operations. To harness the breeding potential of farmed insects, methods to investigate population genetic diversity and structure through population and evolutionary genetic principles are provided as well as tools for monitoring genetic variation and assessing genetic consequences of captive breeding to adequately manage populations. An overview is given on quantitative genetics of farmed insects, how to record phenotypes and pedigrees, estimate genetic parameters, and design optimal breeding programmes. Lastly, the role of functional genetics in insect production is discussed, the biological link between DNA and phenotypic variation, and key to effectively apply genetic improvement strategies through selective breeding. This article identifies knowledge gaps in insect breeding and provides recommendations for application and future research. Major challenges in the field of genetics of farmed insects include how to phenotype large numbers of individual insects over generations; how environmental factors affect

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trait expression, including interaction with genetics; and how to translate results from laboratory settings to mass rearing environments. This article will contribute to further develop the area of genetics of insects for food and feed.

Keywords

demographic inference – insect breeding – population management – review – selection scheme

1 Introduction

Insects have long been a traditional component of the human diet in many regions worldwide, particularly in Africa, Asia, and Central and South America (Chakravarthy *et al.*, 2016; Omuse *et al.*, 2024; van Huis, 2013). Recently, there has also been a rapidly growing interest in insect production for animal feed and human food in the Western world (van Huis *et al.*, 2021, 2025). Whereas traditional edible insects were predominantly beetle larvae, caterpillars, grasshoppers, crickets, and termites (Chakravarthy *et al.*, 2016), novel farmed insects are mostly flies and Tenebrionid beetles. Adults or larvae are either harvested whole or processed for ingredients, such as purified proteins and fats for human food (Bellezza Oddon *et al.*, 2025; Dunkel and Payne, 2016; Hazarika and Kalita, 2023) and animal feed (Heuel *et al.*, 2022b; Stadtlander *et al.*, 2017; van Huis *et al.*, 2025). Nutritional profiles of insects are favourable for omnivorous and carnivorous diets (Heuel *et al.*, 2022a; Makkar *et al.*, 2014) and feeding live insects could be a veritable enrichment component in animal production (Bellezza Oddon *et al.*, 2021). A smaller focus lies on the production of insect components for technical applications (Rehman *et al.*, 2023), for cosmetics and paints (Franco *et al.*, 2022; Triunfo *et al.*, 2021), and the agricultural use of insect frass (Barragán-Fonseca *et al.*, 2022; Poveda, 2021).

The potential for reducing ecological footprints of food chains, for instance, by replacing barely sustainable feed components like soy and fishmeal with insect-based products in livestock and aquaculture production (Heuel *et al.*, 2021; Hua, 2021) is widely acknowledged (e.g. Smetana *et al.*, 2021; van Huis, 2013; van Huis and Oonincx, 2017). Yet, the field faces diverse challenges, e.g. regulatory restrictions in several regions complicate large-scale production (Lähteenmäki-Uutela *et al.*, 2021), and food and feed safety issues (Heuel *et al.*, 2023; Wynants *et al.*, 2019) deriving from waste stream bioconversion (Bosch *et al.*, 2019; Ewusie *et al.*, 2018; Gold *et al.*, 2018). Other questions concern insect nutritional requirements (Oonincx *et al.*, 2025), infections and disease vectoring (Joosten *et al.*, 2020; Vogel *et al.*, 2022), reproduction and welfare under mass rearing conditions (Tomberlin *et al.*, 2025) and data analyses (Smetana *et al.*, 2025). As insect production can only be considered an emerging industry at this point, economic concerns are at the forefront of the stakeholders' considerations. Optimisation of production processes can be approached along various lines, including the technical production facilities and environmental conditions (Coudron *et al.*, 2025; Deruytter *et al.*, 2025), but also genetic improvement through selective breeding. Only very recently is the sector becoming aware of the pervasive and palpable impact of the genetic makeup of insects farmed for food and feed and opportunities for breeding to support the sector's maturation comparable to the state-of-the-art in traditional livestock (Eriksson and Picard, 2021; Hansen *et al.*, 2024b; Jensen *et al.*, 2017; Sellem *et al.*, 2024).

For decades, livestock populations have been tailored to the requirements of their specific production system. Animal breeding has grown into a highly specialised and complex research field covering sophisticated phenotyping and performance testing systems, statistical models for genetic evaluation and prediction in various species, and the integration of genetics and economics into robust breeding programme designs (e.g. Gianola and Rosa, 2015; Miglior *et al.*, 2017; Pérez-Enciso and Steibel, 2021). However, for farmed insects, and especially insects for food and feed, systematic selective breeding is still in its infancy, and its establishment will require a great deal of optimisation. Insect production systems have unique biological and operational characteristics that make the task of genetic improvement particularly challenging. These include the existence of multiple life stages (egg, larva or nymph, pupa, and adult), usually short lifespans, small size of individuals, complex reproductive behaviour and the sheer scale of populations leading to a very poor and/or unpredictable representativeness of a single individual, which complicates the evaluation of individual phenotypes. These challenges demand the development of new paradigms, potentially inspired by aquaculture breeding, where

similar complex systems exist. Adapting selective breeding to insect production also requires amalgamating the different fields of farmed insect genetics to align priorities and achieve a common goal. Researchers and insect breeders currently involved in the field of farmed insect genetics come from a range of backgrounds, each bringing unique perspectives, methodologies, and areas of expertise, which, while enriching, can also create silos and challenges in communication and integration.

The necessary harmonisation of breeding programmes and goals faces the challenge of the diversity of insect species produced for food and feed at present and in the future. Among more than one million insect species described (and many more estimated) (Stork, 2018), numerous are highly promising to explore as food, feed, and technical products (van Huis, 2020). However, the number of insect species used for production purposes is currently restricted to a few dozen (Francuski and Beukeboom, 2020; van Huis and Tomberlin, 2017). For most primary species there is a striking lack of knowledge on their evolutionary history and species-wide population genetic structure (up to complete ignorance of the existence of wild congeners of some production insects). This BugBook article collates available insights for four thematic sections – molecular, evolutionary, quantitative and functional genetics – for the prominent (traditional and novel) insects for food and feed. Examples include the black soldier fly (BSF) (*Hermetia illucens* L.; Diptera: Stratiomyidae), the house fly (*Musca domestica* L.; Diptera: Muscidae), the yellow mealworm (*Tenebrio molitor* L.; Coleoptera: Tenebrionidae), the lesser mealworm (*Alphitobius diaperinus* L.; Coleoptera: Tenebrionidae), the house, the tropical house, and the Mediterranean field cricket (*Acheta domesticus* L., *Gryllodes sigillatus* L., and *Gryllus bimaculatus* De Geer; Orthoptera: Gryllidae), as well as migratory and desert locusts (*Locusta migratoria* L. and *Schistocerca gregaria* Forsskål; Orthoptera: Acrididae). We complement the conceptual framework and relevant approaches for genetics research by several comparatively more detailed or advanced insect examples. These include model insects in the academic field, such as the vinegar fly (*Drosophila melanogaster* L.; Diptera: Drosophilidae), the parasitoid wasp *Nasonia vitripennis* (Walker; Hymenoptera: Pteromalidae) and the red flour beetle (*Tribolium castaneum* L.; Coleoptera: Tenebrionidae), insects farmed for other purposes than food and feed, such as the honeybee (*Apis mellifera* L.; Hymenoptera: Apidae) and the silkworm (*Bombyx mori* L.; Lepidoptera: Bombycidae), but also pest and biocontrol insects.

In this BugBook article, we provide comprehensive overview of genetic principles, processes and tools as well as recommendations for further genetic studies and applications to advance the field of insects for food and feed. We aim to unify knowledge and foster cooperation to accelerate progress in insect breeding programmes and address the unique challenges of insect production systems, ultimately advancing sustainability and productivity. Throughout thematic Sections 2-5 (Figure 1), we review the wider field of insect genetics, ranging from molecular methods commonly used for insect genetic research, to quantitative genetic approaches, to selection of breeding candidates with superior traits. New approaches and methodologies, as well as those established in animal breeding, are critically considered for their application in academic research and the insect industry, along with the identification of specific technological demands, challenges and opportunities. We outline central concepts of population genetics and evaluations of genetic diversity and dynamics of genetic variation in space and time, which is crucial for deciphering hierarchical metapopulation structure across field and production contexts, as well as demographic patterns relevant for inferring domestication processes and conservation strategies. We also discuss the deep knowledge of population structure and evolutionary history required to make informed selection and crossing decisions and effectively manage farmed insect populations in a sustainable manner, as well as biological relationships between genetic variation and functional phenotypic variation. Finally, we discuss conceptual links between the topic areas (Figure 1) to identify interdisciplinary challenges and synergies, and make concluding recommendations for future directions to implement progress in the field of farmed insect genetics.

2 Molecular genetics

A comprehensive understanding of the basic biology and evolutionary history of any given farmed insect is key to inform and improve selective breeding programmes, and ensure high health and quality standards in mass rearing operations. This section describes various molecular genetics and genomics analyses, selection and preparation of samples, and the optimal DNA/RNA extraction methods for addressing specific

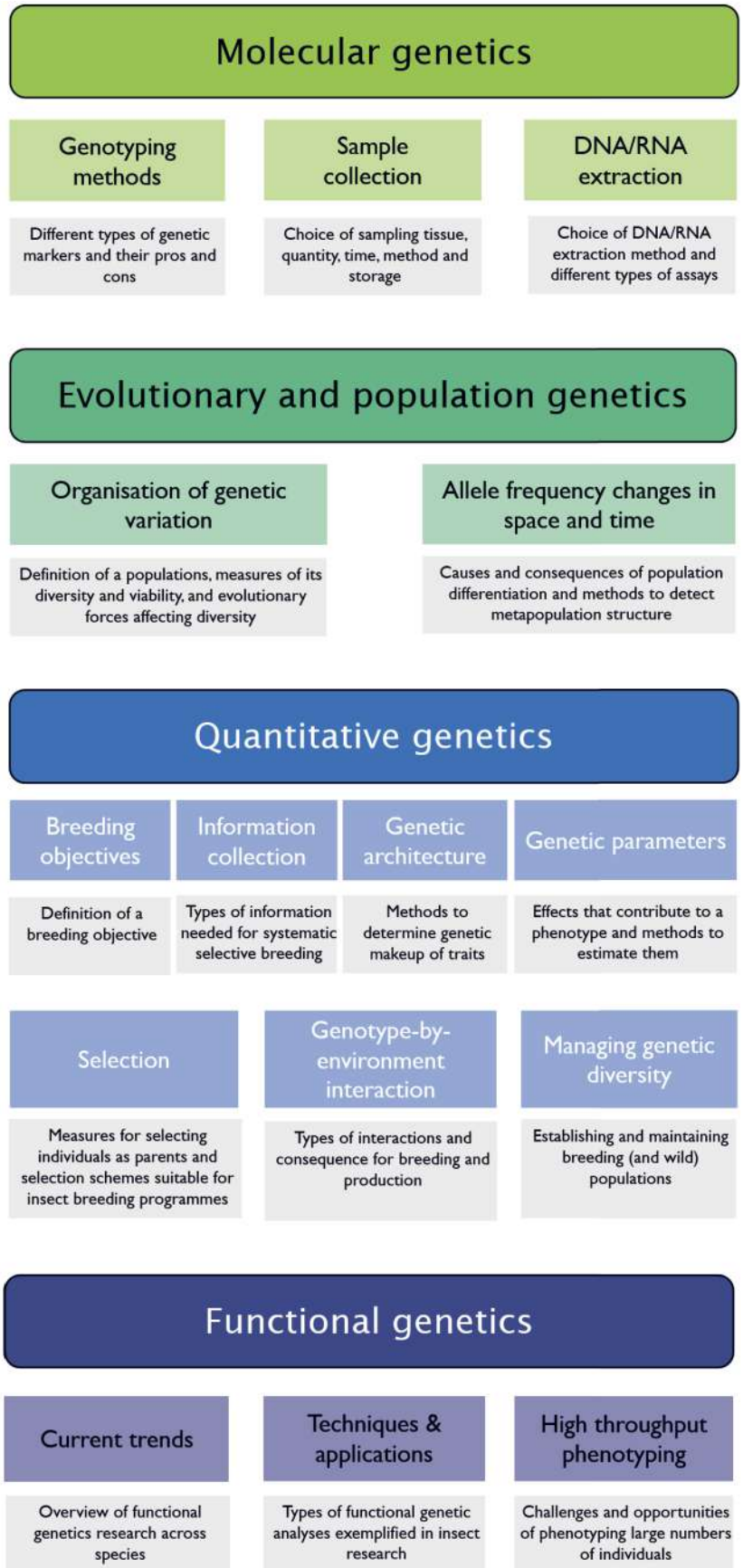

FIGURE 1 Graphical index contextually capturing thematic Sections 2-5.

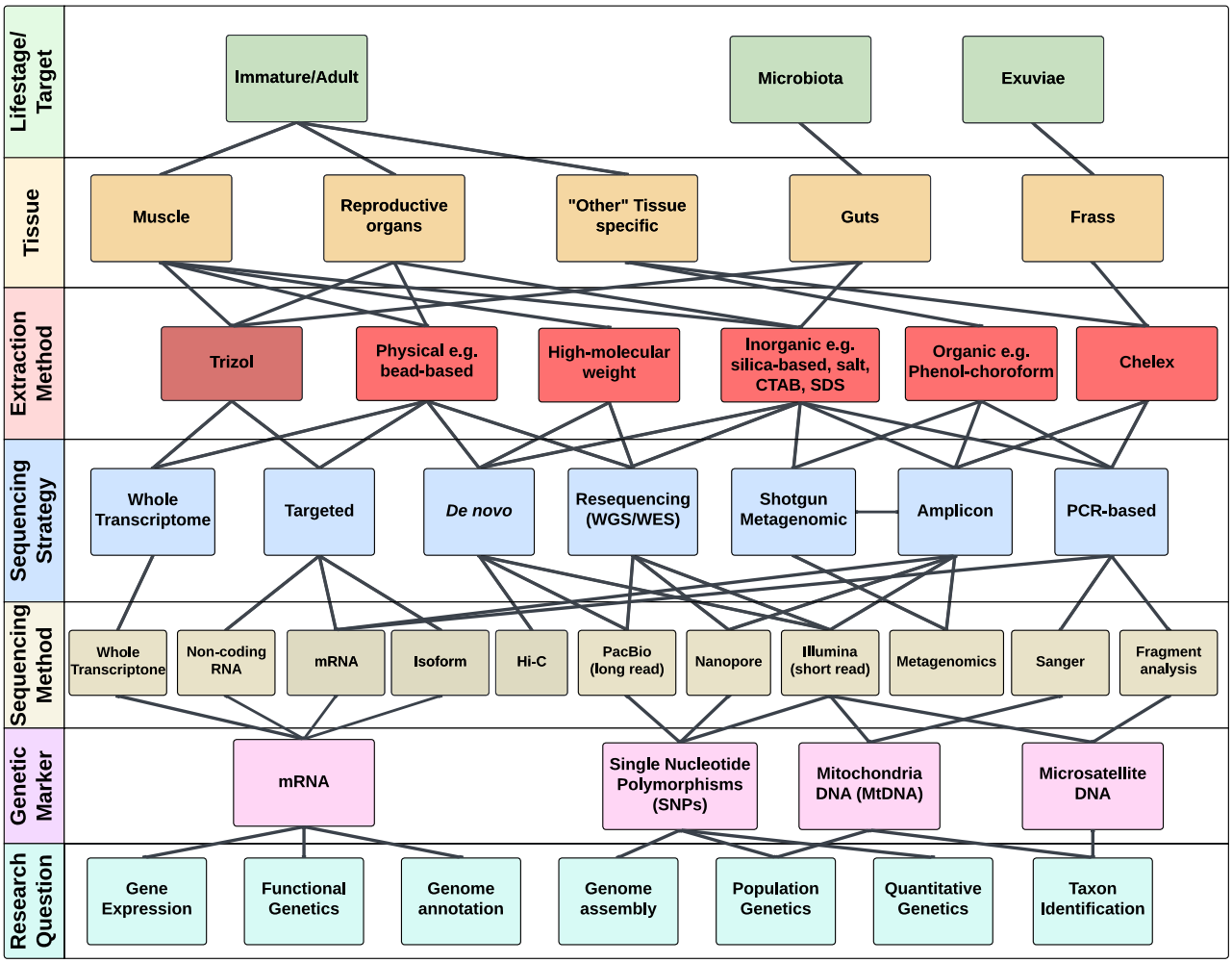

FIGURE 2 Potential bidirectionally interpretable and mutually non-exclusive research pathways to address molecular genetics in insects for food and feed.

questions from barely equipped applied research to large-scale bioinformatics endeavours.

Genotyping methods

The polymerase chain reaction (PCR) is a fundamental technique in modern molecular biology. Delineated by specific primers, it amplifies a target region in the nuclear or mitochondrial genome. PCR facilitates the study of genomic regions from a limited amount of starting material, enabling genetic analysis of virtually any organism without constraints on the sampling environment (Freeland, 2020; Rowe *et al.*, 2017). Genotyping techniques can be categorised into PCR-based and non-PCR-based methods. Within each category, various assays can be performed on mitochondrial DNA (mtDNA), nuclear DNA, or messenger RNA (mRNA) that serves as the intermediate molecule between DNA and proteins. These methods range from simple techniques, such as elucidating evolutionary relationships through PCR-based amplification followed by Sanger sequencing, to the assessment of total genome diversity, variability, and structural variation through whole genome sequencing (Figure 2) (Holliday *et al.*, 2019; Hoy, 2019).

Mitochondrial barcoding

One of the most common, and relatively inexpensive, methods of genotyping is to sequence different regions of mtDNA. The small, circular, double-stranded maternally inherited DNA molecules (approx. 16 kb) are abundant (many copies per cell), stable, suitable for non-invasive sampling, and easily purified for genetic analyses. mtDNA contains coding regions for highly conserved, essential genes involved in energy metabolism (Hwang and Kim, 1999; Wallace and Chalkia, 2013) allowing the design of PCR primers that work universally across entire phyla such as arthropods. Consequently, mtDNA remains the most accessible genetic marker for assessing phylogenetic and phylogeographic relationships (Dowling and Wolff, 2023; Wu *et al.*, 2022). Accurate classification of an organism's evolutionary

origin is crucial, as unresolved taxonomic uncertainties, like unrecognised sibling taxa within a cryptic species complex, would impede the definition of management units necessary for tailored breeding in insect livestock (Section 4).

Typically, short fragments of several hundred base pairs comprising individual genes are sequenced rather than entire mitogenomes, such as the commonly used 'barcode' region (Pentinsaari *et al.*, 2016), which encompasses most of the cytochrome oxidase I (COI). Analysing additional genes within the mitochondria does not always provide significant advantages due to the linked nature of the loci (Sandrock *et al.*, 2011b) even though assembling mitogenomes of farmed insects is affordable (Homchan *et al.*, 2024; Yu *et al.*, 2022). Although COI barcoding is barely useful to address intra-population variation, it can represent a minimal approach to discriminate strains of different origin in comparative studies, as demonstrated in the house fly (Pastor *et al.*, 2014). Further comparison with a comprehensive phylogeography, as available for the BSF and so far capturing approximately 60 distinct COI haplotypes worldwide (Guilliet *et al.*, 2022; Khamis *et al.*, 2020; Nguyen *et al.*, 2023; Pazmiño *et al.*, 2023; Ståhls *et al.*, 2020), is particularly meaningful in this context (see Section 3: Mito-nuclear phylogeographic patterns). Remarkably, BSF haplotypes exhibit divergences of up to 4.9%, which may be indicative of a transition to cryptic taxon delimitations (Hebert *et al.*, 2003; Lavinia *et al.*, 2017), suggesting the need for further investigation (Generalovic *et al.*, 2023). Based on few available studies, divergence in the yellow mealworm remains way shallower, merely reaching 2% (Song *et al.*, 2022). Importantly, all amplified and sequenced genes must be verified as mitochondrial, and not pseudocopies present in the nucleus of many insect species called nuclear mitochondrial DNA segments (NUMTs). This is typically verified via translation of the gene sequence (Hazkani-Covo *et al.*, 2010).

Microsatellites

Microsatellites, synonymously referred to as 'simple sequence repeats (SSR)' or 'short tandem repeats (STR)', are short DNA sequences of 2-6 bp that are repeated few-to-many times. They tend to be widespread in eukaryotic genomes and usually located within non-coding parts such as intergenic or intronic regions. Repetitive motifs experience comparatively high mutation rates at evolutionary scales (Weber and Wong, 1993). Hence, their presumed neutral evolution allows these markers to accumulate high allelic diversity, often comprising dozens of alleles per locus. Conversely, microsatellites frequently reside in 'junk' DNA regions which challenges designing primers within respective flanking regions that are locus-specific and conserved for universal intraspecific amplification. Microsatellites have been and still are widely used for genetic profiling since the 1990s, especially in forensics and paternity testing (Butler, 2014), yet also for population genetics (Section 3) and evolutionary ecology research (Brede *et al.*, 2006; Buellesbach *et al.*, 2023; Sandrock *et al.*, 2007).

Using previously established microsatellite markers is particularly useful if standardised reference data sets are openly accessed for subsequent cross-study comparison. Microsatellites are for example available for BSF (Kaya *et al.*, 2021; Rhode *et al.*, 2020). If no published markers exist, the basic development of new microsatellites can be inexpensively accomplished by generating whole genome sequence reads. Using available pipelines (e.g. msatcommander, (Faircloth, 2008)), reads can be screened for repetitive motifs complemented by adequate primer design swiftly yielding many new microsatellite markers (Abe and Pannebakker, 2017). Next, establishing and validating a robust microsatellite marker set involves screening for meaningful polymorphisms as well as evaluating possibly segregating "null alleles". These are alleles for which amplification fails due to polymorphisms in a given primer binding region. Such drop out alleles are problematic, and their frequencies may vary among populations. Therefore, newly developed microsatellite loci require a thorough population survey to assess the quality of the designed primers in their ability to amplify across populations. If primer re-design is no option (e.g. due to other amplification artefacts), candidate markers standing out because of null allele homozygotes should be excluded from sets of routinely implemented loci. Further, hypervariable as well as barely polymorphic loci both have their merits, depending on relative scopes to resolve relatedness within and across populations (Section 3).

Microsatellite analysis basically uses conventional PCR amplification. For convenience, multiple loci may be co-amplified in multiplex PCR reactions, if annealing temperatures of individual primers allow or were co-designed for that accordingly. It is imperative to label the forward primers of markers that amplify sequences of possibly overlapping fragment length using (up to six) different fluorescent dyes, and purposive primer design in the flanking regions ensures non-overlapping ranges of finally amplified sequences when the same dye is used. In this way, co-amplification of 5 to 8 loci

is readily possible within in one reaction (Sandrock *et al.*, 2010) but combining up to 24 loci e.g. in human genotyping kits is feasible (Williams *et al.*, 2023). Following the amplification of polymorphic microsatellite loci via PCR, allelic discrimination is performed based on capillary electrophoresis in relation to an internal size standard. This allows for the resolution of fragments that differ by 1 nucleotide or more. Several software tools exist to handle the raw data and visualise fluorescence signals that effectively capture fragment length (e.g. GeneMapper (Thermo Fisher Scientific, Waltham, MA, USA) or Geneious (Dotmatics, Boston, MA, USA)). In diploid genotypes, the sizes of the fragments allow to differentiate between homozygotes and heterozygotes, i.e. sizes of the two alleles are either the same or different, respectively. Not to be generalised, amplification of repetitive motifs can exhibit stutter signals, which requires careful allele calling for some markers.

Alternatively, sequence-based microsatellite genotyping (SSRseq) combines next-generation sequencing techniques with multiplex PCR amplified microsatellite loci (Lepais *et al.*, 2020; Zhan *et al.*, 2017). Because this yields direct access to allele sequences, up to 60 loci can simultaneously be amplified. SSRseq also resolves the issue of homoplasy, where alleles that are genotyped to the same size using electrophoresis represent two alleles due to masked SNPs or indel variation. One requirement of SSRseq is reference genome data, which is currently available for many insects for food and feed, including the BSF (Cai *et al.*, 2024; Costagli *et al.*, 2024; Generalovic *et al.*, 2021; Zhan *et al.*, 2020); yellow mealworm (Eleftheriou *et al.*, 2022a; Eriksson and Picard, 2021; Kaur *et al.*, 2023; Oppert *et al.*, 2023); house cricket (Dossey *et al.*, 2023); house fly (Scott *et al.*, 2014); migratory locust (Wang *et al.*, 2014); or can be newly generated from low coverage genome sequences. By means of efficient processing pipelines and automated data analysis, this approach can be readily normalised across laboratories to yield high-quality microsatellite data, supplemented with SNP and indel variation, for many individuals (Choi *et al.*, 2022; Lepais *et al.*, 2020).

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are alternative markers for genotyping. In contrast to the multiallelic nature of microsatellites, most SNPs are biallellic. Each SNP locus therefore provides less information per locus than a single microsatellite (e.g. Liu *et al.*, 2017), but SNPs have a much higher density in the genome and readily extend into relevant coding regions, making them more informative with regards to genome coverage and locus representation (Thalamuthu *et al.*, 2005). Furthermore, as SNPs are mutationally more stable, it makes them less prone to homoplasy that might confound genetic analysis (Zimmerman *et al.*, 2020). With the recent technical ease of high-throughput genotyping technologies, SNPs are becoming the markers of choice in genomic applications, such as QTL-mapping, genome-wide association studies (GWAS), marker-assisted selection, genomic prediction, and resolving evolutionary relationships (Sections 3 and 4).

Several methods exist for genotyping SNPs, the choice of which depends on numbers of samples and SNPs required. For genotyping a limited number of SNPs in a limited number of samples, Sanger sequencing of PCR amplified regions of up to 1000 base pairs long is most often used (Hawkins, 2017). In contrast to fragment analysis of microsatellites, Sanger sequencing of PCR amplicons provides complete information (all polymorphisms as well as heterozygous sites). If only a few SNPs need to be genotyped, for instance those known to be linked to a phenotype from previous studies (Section 4), methods that directly target a specific SNP are more useful. Of these, both TaqMan probes and Kompetitive Allele-Specific PCR (KASP) assays are frequently used (He *et al.*, 2014; Woodward, 2014). For genotyping a single bi-allelic SNP, TaqMan uses two allele-specific probes labelled with different fluorescent dyes, each hybridising to a different allele in quantitative PCR (qPCR) also known as real-time PCR (RT-PCR) amplification (see Section 2: PCR-based RNA assays assays). Alleles are then determined by measuring the intensity of the fluorescent dyes as they bind to the template. While sensitive and reliable, TaqMan technology is limited by the use of the specialised allele-specific probes (Woodward, 2014). Conversely, KASP uses unlabelled allele-specific primers with a unique tail sequence that binds to different fluorescent dyes, allowing the determination of genotypes based on end-point PCR products (He *et al.*, 2014). It is more cost-effective than TaqMan, and therefore suitable for high-throughput analyses typically required by breeding programmes.

Genotyping a larger number of SNPs distributed throughout the genome, particularly for GWAS, typically requires either next-generation sequencing (NGS) methods (e.g. low-coverage Illumina sequencing (Lou *et al.*, 2021)), targeted enrichment strategies using a set of DNA probes for the targeted SNPs (then sequenced using Illumina technologies), or pre-made microarray systems including required SNPs. Although the bioinformatics approaches differ between these methods,

all depend on genotypes being compared to reference genomes. Due to the large sample sizes needed to associate specific traits with populations in GWAS, using NGS can be both costly and time-consuming. While SNP arrays are also expensive, they are generally more practical for such large-scale studies. Currently, there is only one high density genotyping array dedicated to farmed insects, which covers 679 205 SNPs and over 99% of the yellow mealworm genome (Axiom® YNS_Mol1).

There are also 'in-between methods' that focus on sequencing only genome subsets, rather than the entire genome. Examples include restriction-site associated DNA sequencing (RADseq), which sequences only the fragments near restriction enzyme sites, and exome sequencing, which targets only the coding regions of the genome (Zhang *et al.*, 2024). Alternatively, very low coverage sequencing of an entire population can be used, or alternatively pooled sequencing (Pool-seq) (Donkpegan *et al.*, 2022; Gmel *et al.*, 2023) for applications that do not require individual genotype information. While these methods are excellent for population differentiation by leveraging existing nucleotide diversity (Fuentes-Pardo and Ruzzante, 2017), they often lack the resolution needed for trait associations and other industrially important questions (i.e. GWAS). This is because most complex traits are polygenic, with each gene typically having a low effect (Section 4), necessitating massive sample sizes to detect rare and often subtle signals in the genome with clearly defined genotypes not typically generated from low coverage SNPs.

Finally, more advanced methods for understanding genetic diversity and its correlation with phenotypes increasingly rely on deep sequencing, typically applied to a smaller number of samples. Pangenomics represents the next frontier, enabling not only the association of nucleotide diversity across populations, but also the assessment of the role of structural variants in genome organisation and phenotypes (Tong *et al.*, 2022). These methods require a substantial amount of high-quality DNA, and in some cases, such as with chromatin-capture techniques like Hi-C for scaffolding (Yamaguchi *et al.*, 2021), fresh tissue is needed to capture subtle yet informative associations. Currently, both PacBio and Nanopore sequencing technologies generate long reads (>10kb) that can detect smaller structural variants, while Hi-C can capture long-range structural variants such as inversions, insertions, and duplications (Figure 2).

We end this section with the following recommendations. Next to basic COI barcoding, microsatellite genotyping is cheap, quick and reliable to characterise and monitor population genetic diversity indices or assign individuals to genetic clusters or admixed ancestry to inform experimental and breeding designs (Section 3). As an extension, the future goal, however, is to validate and implement high to medium density SNP arrays when starting the selection scheme and subsequently monitor generations in industrial breeding. SNP genotyping or sequencing is required to highlight interesting SNPs, linked to traits of interest by means of GWAS, and to design small density arrays or SNP panels (low-cost SNP sequencing) aiming to convey a genomic selection scheme (Section 4). SNP panels and the use of imputation, however, are sensitive to the reference population. This can be problematic without long range information on the presence of structural variants contributing to the loss of amplification of alleles or haplotypic linkage of various SNPs (Kapun *et al.*, 2021).

Sample collection

Secure sampling storage

Reliable genotyping and genomic results necessitate sampling and storage conditions tailored to the intended use of extracted materials. DNA can be extracted from all insect stages, including hemi- and holometabolous species. When collecting specimens for subsequent molecular methods, their initial storage conditions, if not being used immediately, are critical to downstream success. The optimal storage option for any insect at any stage is to flash freeze in liquid nitrogen and store at −80 °C environment. The next most effective method is to euthanise the insect as rapidly as possible (freezing) and then store it in a nucleic acid storage solution (e.g. RNAlater or a similar chemical solution) or >70% ethanol, preferably at lower temperatures (4 °C or −20 °C). Ethanol dehydrates the specimens, effectively neutralising the enzymatic activities that would degrade nucleic acids; however, it is crucial that the solution remain >70%. Thus, after 24-48 hours of storage, it is important to replace the ethanol anew, given that the original solution will be diluted with moisture from the insects, and particularly larvae. Air-dried or pinned specimens can be utilised for DNA extractions, but the DNA can be degraded, likely yielding only small amplicons (Fong *et al.*, 2023). While DNA is constant across life stages, tissue and environmental conditions, mRNA is much more sensitive and more volatile depending on the context. The expression of genes depends on the exact conditions prior to sampling. Some genes, e.g. heat shock genes, respond by up- or down-regulation within minutes of changed environmental conditions (Sørensen *et al.*, 2003). Furthermore,

RNA is much faster degraded than DNA. Therefore, sampling design is important for the samples to serve the purpose of the study, and fast handling and storage (preferably at –80 °C or in dedicated solutions designed to preserve RNA) is crucial to ensure integrity of the sample.

Choice of life stage and tissue

The choice of life stage and tissue type will also impact downstream analyses. Vice versa, to pursue a certain research question and successive methodological pathways, contingent upon accessible technology, investigators ideally define specific targets, as schematically illustrated in Figure 2. If generating extracts for individual gene sequencing (e.g. mtDNA COI), then a very small amount of DNA is needed. If NGS methods (PCR-free) are desired, then a much higher concentration is needed (>100 ng/µl). For example, approx. 50 yellow mealworms eggs must be pooled to obtain between 20 and 50 ng/µl of DNA. This is an important factor to consider when standardising DNA extraction protocols. From larval, pupal, or adult stages, tissue quantity is largely sufficient to obtain high DNA quantity; however, the tissue source plays a pivotal role for DNA quality and quantity when working with small amounts of starting material. As larvae get larger, fat body tissue becomes increasingly extensive throughout the body, which results in lower quantity and often quality of DNA due to the difficulty of separating lipids during the extraction procedure. Adult tissue, specifically muscle tissue, is an excellent tissue type, and should be considered whenever possible, especially for genome-wide sequencing methods. It is necessary to use a DNA source with sufficient cell density from which a non-negligible quantity can be sampled without risks.

Design and timing

The sampling design for collecting insects is crucial for accurately assessing genetic variation in population studies, whether the focus is on wild or farmed populations (Meirmans, 2015). Representative sampling (timing of collection and numbers of individuals) avoids bias, as well as inadvertently pooling subpopulations that have different allele frequencies leading to misinterpretation of genetic structure (Section 3). This can arise if samples are collected from different developmental stages at the same time and place, or if samples from nearby sites are pooled together. Additionally, special care is needed in farmed populations where genetic drift can become significant, particularly if batch production involves discrete, non-overlapping generations. For Evolve and Resequencing studies (Section 4), recommendations suggest sampling over enough generations to detect selection, with simulations indicating that 10-20 generations are often optimal. This allows for the accumulation of genetic changes due to selection while controlling for drift. Replication is also key in these studies, as it helps distinguish true selective responses from random variation. Moreover, the strength of selection should be carefully considered, as stronger selection can lead to more detectable changes over fewer generations at the expense of an elevated risk of genetic drift and hitch-hiking of genetically linked loci, while weaker selection may require longer observation periods to discern its effects but with less drift due to higher effective population size (see Section 3: Population differentiation and its causes) and allow more recombination.

Non-destructive sampling

Current methods for DNA collection and extraction from insects are destructive, as the most common techniques macerate entire individuals (Figure 2) (Huanca-Mamani *et al.*, 2015). Indeed, two limiting factors in processing non-destructive extractions remain; the limited amount of extracted DNA derived from the small tissue quantities and the effects on the insect that likely affects either the phenotype of breeding candidates (see Section 4: Phenotyping), and/or fitness. Few protocols have been developed to extract insect DNA from frass (Kidd *et al.*, 2003) and exuviae (Kranzfelder *et al.*, 2016), but this method cannot identify individuals, resulting in a 'population' sample. This approach can be particularly useful for insects at the larval stage, where large quantities of frass and exoskeleton sheds are produced. However, the quality of DNA suffers from degradation by nucleases when collected in the field resulting in insufficient DNA quantity (Peng *et al.*, 2018). High-quality DNA was also extracted by sampling 2-3 mm^2 wings clips from red flour beetles (Châline *et al.*, 2004), and honeybee queens, as well as from their exuviae and faeces (Bubnič *et al.*, 2020). Legs seem interesting due to their large quantity of muscle tissue as a suitable source of DNA (Ožana *et al.*, 2020). Another promising and quantitatively efficient DNA source is haemolymph. However, collecting the latter two could be particularly difficult without huge individual impairments, e.g. through immune responses affecting overall performance (Vogel *et al.*, 2022).

DNA/RNA extraction methodology

Sample processing

Methods for obtaining nucleic acids depend on the intended downstream applications, with each method presenting its own advantages and disadvantages in terms of ease of procedure, cost, and quality of the extracted nucleic acids (Figure 2). In some cases, kit-based methods are used, which provide all the necessary components, while in other cases, chemical-based methods are employed, which require the purchase of all components.

DNA/RNA extraction, in particular, involves three distinct steps: cell membrane and nuclear membrane lysis, stabilisation of DNA/RNA, and precipitation and washing. To effectively isolate DNA/RNA, it is necessary to break down the cell membrane and nuclear membrane, which are composed of roughly equal parts glycoproteins and lipids. To liberate DNA/RNA from chitinous materials such as legs and wings, buffers containing relatively high levels of dithiothreitol (DTT), proteinase K, and detergent are typically used. Following isolation, the next step is to remove contaminants without compromising the stability of the DNA/RNA. DNA/RNA is then precipitated and washed to remove other protein debris.

It is important to note that DNA extraction methods can vary significantly from one another, with broad categorisation into chemical vs silica-based and mechanical methods. Chemical methods are further divided into organic extraction techniques, such as the phenol-chloroform-isoamyl alcohol (PCI) method, and inorganic extraction techniques, including cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS) extraction, salting out, and solid-phase extraction techniques. Finally, physical extraction includes magnetic bead-based methods and silica-based spin column extractions. Figure 2 outlines available and recommended DNA extraction methods based on the desired analyses/outcomes.

PCR-based assays

PCR-based methods (mtDNA and other DNA sequencing as well as microsatellite and SNP genotyping), require uninhibited DNA. Traditional, less expensive, and time-saving chemical-based methods are suitable for these applications, as they are effective in many sample assays. The quickest method, which utilises Chelex beads to chelate DNA from various materials and lyses cells using heat, is not necessarily the most cost-effective. The yield of small DNA fragments is not ideal for many applications. While Chelex extraction may outperform other methods in terms of DNA quantity, purity is often problematic, which, in conjunction with single-stranded DNA, negatively affects storage stability, except for shorter fragments up to a few hundred base pairs (Al-Griw *et al.*, 2017). Despite these limitations, Chelex extraction is effective in removing PCR inhibitors and is commonly used in PCR-based methods, such as mtDNA, microsatellite, or amplicon sequencing. Alternative methods include chemical extraction procedures, such as PCI and CTAB, as well as 'salting out' methods using a high concentration of salts. PCI extraction uses organic solvents to separate DNA from proteins and other cellular debris, yielding high quality DNA. However, it is labour-intensive, and requires careful handling of hazardous chemicals, and often introduces residual phenol that can inhibit downstream PCR analysis. CTAB and other salting out methods are less toxic, but yield can vary widely depending on the amount of tissue used (Figure 2).

Native genomic DNA assays

For both whole genome sequencing (WGS) and whole exome sequencing (WES) it is important to start with high quality starting material, which is fresh or flash-frozen at ultra-low temperatures (–80 °C) which effectively rules out frass and exuviae as a reliable source material for these purposes (Fuentes-Pardo and Ruzzante, 2017). Several methods are commonly used for DNA extraction, each with their own advantages and disadvantages. A traditional method is the PCI extraction. Further, silica-based column extraction kits, such as Qiagen DNeasy Blood and Tissue Kit (Qiagen) or the Monarch PCR and DNA Clean-up Kit (New England Biolabs) are widely used and are usually the first method of choice for beginners due to their simplicity, efficiency, and minimised contaminants carrying through. However, yields can vary depending on the sample type and specific kit used. Magnetic bead-based extraction methods are another popular method, especially given their propensity to be automated, resulting in high purity extractions. However, residues from the magnetic beads can interfere with library preparation and sequencing procedures.

Each of these methods can be tailored to specific sample types, downstream applications, and sequencing methodology, making them versatile tools in genomic research. For example, if long-read sequences are desired (i.e. PacBio HiFi or ONT Minion), kits and other methods exist to ensure gentle digestion and limited mechanical shearing to ensure long intact fragments of DNA (Figure 2).

PCR-based RNA assays

RNA extractions required for qPCR and PCR-based transcriptomics are undeniably more complicated and require extensive planning due to the prevalence of RNases in the environment. The initial step involves collecting the desired tissue and ensuring it is free of RNase activity, which involves sterilising instruments with a cleanser like RNase Away or other decontamination reagents. Once the sample is prepared, the procedure is remarkably similar to DNA extraction, with options to use solid-phase methods (silica-based kits), acid phenol methods like Trizol, and magnetic beads methods. As for DNA extraction, dedicated kits are commercially available from all main supplies, e.g. RNeasy Kit (Qiagen) or E.Z.N.A.® Total RNA Kit (Omega Bio-tek). Additionally, a DNase treatment is often added to the purified RNA extractions to eliminate any contaminating DNA fragments. To assess specific changes in expression for specific genes using qPCR on mRNA transcripts, cheaper methods that allow for the analysis of many samples with replication, such as Trizol or automated magnetic bead systems, would suffice (Figure 2). Alternatively, RNA can be converted to complementary DNA (cDNA) using reverse transcription (e.g. Omniscript RT, Qiagen) to generate more stable templates for downstream qPCR gene expression analyses (Section 5). For standard short-read based RNAseq experiments, silica-based kits, Trizol, and magnetic beads all work well. If the goal is to sequence only mRNA, using a method that enriches for polyadenylated RNA (i.e. using oligo (dT) beads) or depletion of rRNA is necessary. Newer technologies have been developed that remove the need for RNA extraction and instead take the tissue directly to qPCR (e.g. Scientific's Cells-to-Ct kit, ThermoFisher). Although efficient, these methods are generally more costly (Section 5).

DNA/RNA-protein-based assays

The interaction of DNA/RNA-protein complexes (without altering the DNA/RNA), i.e. epigenetic regulation, should also be considered important for the future of the insects as food and feed industry. Modification of histone complexes causes structural changes that regulate gene expression and repression (Glastad *et al.*, 2019). Therefore, phenotypes of interest, maintained by simple or complex gene regulatory networks, may not differ between populations whilst the epigenetic regulatory control may vary (see Section 5: Gene expression analysis). Typically, bead- and column-based extraction methods are required (Figure 2). Unlike DNA/RNA extractions, the ability to efficiently extract histone complexes requires specialised equipment for optimised fragmentation via sonication. Several sequencing methods exist for studying the epigenome e.g. histone modifications (ChIP-seq), DNA Methylation (MeDIP), chromatin conformation (Hi-C), chromatin accessibility (ATAC-seq), etc., as reviewed extensively by Li (2021).

3 Evolutionary and population genetics

While arguably one of the most important aspects for understanding species biology and implementing effective genetic management strategies, defining a population is not a trivial task. Characteristics such as its effective population size, genotype and allele frequency distributions, and the degree of linkage disequilibrium determine the extent of phenotypic variation within the population, and thus its potential for adaptive evolution, extinction risk, and 'susceptibility' to stochastic processes (i.e. genetic drift) under natural and man-made selection pressures. Here, we outline why and how to assess these parameters, how to evaluate single- and multi-population structure, and how to reconstruct evolutionary relationships and demographic history.

For a summary of relevant measures and tools for evolutionary and population genetics analyses we refer to dedicated reviews, including Excoffier and Heckel (2006), Bourgeois and Warren (2021), Saravanan *et al.* (2020), Casillas and Barbadilla (2017) and Sethuraman *et al.* (2020), that are also collated in Table A1 in the Supplementary material.

Basic organisation of genetic variation

The population concept

Although one of the central concepts in biology, the definition of a population depends on the context. Two broad conceptual understandings of biological populations exist: (i) the ecological view that defines a population as a group of organisms of the same species that co-inhabit a space in time; and (ii) the evolutionary view that emphasises the reproductive cohesion of a group of organisms (Waples and Gaggiotti, 2006). Through the application of assisted reproductive technologies (e.g. artificial insemination and embryo transfer) and the continuous displacement of farmed species by human activities, these two definitions have become quite separated from each other, because individuals isolated by both geographical space and time can still contribute to a single gene pool. Throughout this BugBook article, the term population refers to units of individuals that are genetically and spatially related, either natural pop-

ulations in a certain geographic location or managed breeding populations including production stocks and laboratory strains, and that together may form a genetically and otherwise subdivided metapopulation.

Effective population size (N_e)

In population genetics, a distinction is made between the census population size (N_c), the estimated 'absolute' number of individuals in a biological population, and the effective population size (N_e), the number of theoretical, genetically distinct individuals that contribute to the next generation (Frankham *et al.*, 2004). As such, the N_e of a population is a critical parameter determining the strength of evolutionary forces acting on that population (see Section 3: Evolutionary forces) and is in most cases much smaller than N_c (Waples and Gaggiotti, 2006). Populations with smaller N_e harbour reduced genetic diversity due to inbreeding and allelic loss by random drift, making such populations susceptible to inbreeding depression, low evolvability, and eventual extinction (Hedrick and Garcia-Dorado, 2016; Palstra and Ruzzante, 2008). Estimating N_e is important for insect farming operations to ensure that (i) founding populations are large enough to sustain a robust breeding pool, (ii) the population remains viable and fit over successive generations, and (iii) sufficient genetic variation persists in the population to enable response to selection (e.g. Hull *et al.*, 2024; Rhode *et al.*, 2020).

Unfortunately, N_e is a notoriously difficult parameter to estimate, as it depends strongly on demographic parameters, including the harmonic mean of N_c over successive generations, sex ratios, and varied reproductive success of mate-pairs. As no single method considers all demographic factors, true N_e is believed to be often overestimated (Luikart *et al.*, 2010). With the advent of molecular genetics and the use of DNA markers (Section 2), N_e is increasingly estimated using molecular approaches (Hohenlohe *et al.*, 2021). These molecular approaches can be grouped into two broad concepts: (i) inbreeding N_e (N_{ei}) and (ii) variance N_e (N_{ev}) (Luikart *et al.*, 2010) based, wherein N_{ei} considers primarily the presence of heterozygosity within a population, and N_{ev} considers the change in allele frequencies over generations. Due to the temporal component, the N_{ev} approach is particularly sensitive to the early detection of loss of genetic diversity and gives an approximation of short-term N_e in a population. Due to their relatively short lifecycles and rapid generational turnover, insects overcome one of the major limitations of computing N_e that require two sampling timepoints, such as N_{ev} (e.g. Rhode *et al.*, 2020). However, as levels of heterozygosity usually only stabilise in the generations following a bottleneck event, N_{ev} can still be considered superior to N_{ei} for giving an indication of mid- to long-term N_e. For estimating N_e from a single sample, the linkage disequilibrium method (LD-N_e) has been shown to be the most robust (Waples, 2024; Waples and Do, 2010). As a population becomes more inbred and thus more homozygous, LD between loci increases in inverse proportion to N_e (e.g. Kaya *et al.*, 2021).

Genetic equilibrium and diversity

Measurements of genetic diversity are important for understanding the genetic health of a population. This is especially true in managed populations with short generation intervals, such as insects, which can experience rapid and irreversible loss of diversity (e.g. Rhode *et al.*, 2020). Yet this also applies to their wild congeners, whose ephemeral habitats might influence population dynamics (e.g. DiLeo *et al.*, 2024). The two primary measures of genetic diversity are allelic diversity and heterozygosity (Greenbaum *et al.*, 2014).

Allelic diversity is defined by the number of alleles present (A_n), which can be impacted by the number and nature of the genetic markers assessed (Section 2). It is reported as numbers of alleles per locus, total alleles across loci, or average number of alleles over a set of loci. Unfortunately, this parameter is subject to bias in sample size – the more individuals, the more rare or low frequency alleles will be detected, meaning that results cannot be directly compared between samples of different sizes. To mitigate this, effective number of alleles (A_e) and allelic richness (A_r) have been developed to correct for rare alleles and allow comparison of estimates across samples. Standardisation is hereby often based on the sample containing the fewest individuals (e.g. Kaya *et al.*, 2021). Other commonly used estimates of 'allelic' diversity include nucleotide diversity that measures the fraction of mismatches between pairs of DNA sequences drawn from the same population (Korunes and Samuk, 2021), and haplotypic diversity that exploits LD across a number of SNPs (e.g. Alburaki *et al.*, 2023).

Heterozygosity measures how alleles segregate into genotypes. For a locus harbouring two alleles, it reaches its maximum at 0.5 if both alleles segregate at equal frequency. With more than two alleles, expected heterozygosity is calculated as one minus the sum of all squared allele frequencies, so generally the more alleles at a locus, the more heterozygosity is expected. In a population that is in Hardy-Weinberg Equilibrium (HWE), where no evolutionary forces act on a particu-

lar locus, each allele in the gene pool should have equal (frequency-dependent) probability to combine with any other allele in the gene pool in subsequent diploid generations (Frankham *et al.*, 2004). This equates to random mating, and mathematically to allele and genotype frequencies remaining at equilibrium after one generation (Mayo, 2008). This measure is called the expected heterozygosity (H_{exp}) and is one of the primary statistics for evaluating evolutionary potential of a population due to its more universal algebraic accessibility compared to allelic richness (Frankham *et al.*, 2004). When non-random mating occurs in a population, including selection subsets of individuals for breeding purposes (Section 4), deviation from expected genotypic frequencies (under HWE) can lead to inflated or deflated heterozygosity estimates. This is captured by the observed heterozygosity (H_{obs}), which is directly determined from counts of the genotypic data. Deviation from HWE is most frequently measured using *F*-statistics, that is fixation indices (Wright, 1965). One of the fixation indices, F_{IS}, quantifies the relationship between observed and expected heterozygosity within a population as $F_{IS} = (H_{exp} - H_{obs})/H_{exp}$, and gives an indication of hetero- or homozygous excess or deficiency. Capturing potential heterozyous deficiency, the F_{IS}-value is also often interpreted as a molecular analogue of the inbreeding coefficient, but should be done so with caution (e.g. Hoffmann *et al.*, 2021).

Based on H_{obs} compared to H_{exp} for individual or combined loci, we can formulate the inbreeding coefficient (*F*) of (i) an individual (I) relative to the total (T) population (F_{IT}); (ii) an individual relative to the subpopulation (F_{IS}); and (iii) a subpopulation compared to the total population (F_{ST}); and $1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$ (Conner and Hartl, 2004). The *F*-statistics are thus of high importance for population genetics analyses and can be conceptually extended to identify population subdivision and metapopulation structure (see Section 3: Detecting (meta)population (sub)structure).

A population in equilibrium will have an *F*-value ≈ 0, where *F* is defined as the inbreeding coefficient of the relative hierarchical population structure (F_{IS} for within subpopulations and F_{ST} between sub-populations). This ultimately measures deficiency or excess of observed heterozygous genotypes across loci, relative to the expected heterozygosity under random mating. Heterozygous deficiency ($H_{exp} > H_{obs}$) leads to positive *F*-values, possibly due to positive assortative mating, where individuals with similar genotypes mate more frequently, and/or inbreeding. Negative *F*-values through excess of heterozygosity compared to random mating can eventually result from negative assortative mating or outcrossing of individuals from genetically distinct populations.

At the individual level, classical inbreeding coefficients describe the probability that two alleles at any locus are identical by descent (Hartl, 2020). When based on pedigree evaluation (F_{PED}), the coefficients are calculated using Wright's formulae and under the assumption of a 0.5 chance of inheritance of each allele between generations. However, pedigree tracking in insects is challenging, especially the paternal side, due to their mating systems. Alternatively, *F* may then be inferred from genotypic frequency data or the length of runs of homozygosity (F_{ROH}) across the genome (Gmel *et al.*, 2023; Kanaka *et al.*, 2023; Purfield *et al.*, 2012; Rodríguez-Ramilo *et al.*, 2019).

Evolutionary forces

Beyond characterising patterns of genetic diversity, understanding the evolutionary processes is pivotal for successfully managing wild and farmed populations. These processes involve a complex interplay of forces (De Meeûs *et al.*, 2007), which can be broadly classified as (i) demographic, which relate to effective population size changes, mating systems, and gene flow, and (ii) locus-specific, which include uneven mutation and recombination rates along the genome, as well as selection. Demographic forces are expected to entail genome-wide impacts on diversity and can be considered neutral background variation (Casillas and Barbadilla, 2017; Lewontin and Krakauer, 1973). Locus-specific evolutionary forces, however, generate region-specific patterns of diversity.

Evolution is defined as the change in allele frequencies within a population, induced by the evolutionary forces. Furthermore, in the absence of evolutionary forces, a population is considered ideal (i.e. infinite size, isolated, panmictic reproduction, with no selection or mutation) and remains in HWE. However, in reality, all populations are influenced by the four evolutionary forces: mutation, genetic drift, migration, and selection.

Mutation is the ultimate source of new genetic variation (Loewe and Hill, 2010). Heritable mutations occur in the germ line, with the introduction of errors during DNA replication (i.e. substitution, deletion, or insertion of nucleotides). As fidelity in DNA replication is important for survival, mutation rates are extremely small. Moreover, the mutation rate is variable along the genome, between individuals, populations and species. Chromosomal rearrangements also represent a form of mutation, and particularly recombination shuffles pre-

existing genetic variation within each population, creating new allelic combinations, potentially leading to phenotypic variation.

According to the neutral theory of evolution (e.g. see Casillas and Barbadilla, 2017), the fate of mutations is largely determined by genetic drift, i.e. the unpredictable, random changes in allele frequencies. Genetic drift depends on N_e (Kimura, 1968) and can lead to either random loss or fixation of some variants (Masel, 2011), and generally to reduced genetic diversity. Particularly small populations are prone to strong drift that can lead to population decline and extinction due to a higher risk of randomly fixing deleterious alleles (Smith and Haigh, 1974).

Low levels of genetic diversity in a population can be rescued by migration that causes gene flow from external individuals, i.e. transferring alleles from one population to another. Even relatively small numbers of migrants per generation can equalize allele frequencies between the source and the sink populations, and result in an effectively interbreeding panmictic population. In that sense, gene flow can counteract genetic drift by increasing N_e. In natural populations, migration is constrained by geographic distances or physical barriers. Populations in closer proximity exchange individuals more frequently, and accordingly, tend to be more closely related. Conversely, captive populations are maintained in isolation and experience little to no gene flow from outside. Combined with elevated levels of drift due to reduced N_e, these populations can rapidly diverge from their source populations, thereby facilitating overall formation of population genetic structure (see Section 3: Genetic clustering, admixture and introgressive hybridisation).

Both migration and genetic drift affect the establishment of new populations by a limited number of individuals from the original population, which is considered a founder effect (Hartl, 2020). Controlling for the putative impact of such population bottlenecks is highly relevant for maintaining variation in populations of insects farmed for food and feed, which usually only contain a fraction of the diversity present in their wild counterparts, with possible health consequences as increased susceptibility to pathogens (Croze *et al.*, 2016).

Selection acts on genetic variation through the fitness of individual phenotypes, according to their reproductive success in particular environments. To enable selection to act on a trait (natural or artificial), the trait must exhibit phenotypic variation within the population that to some extent must stem from genetic variation between individuals (Falconer and MacKay, 1996). New genetic variants introduced through mutation or migration are generally neutral, less frequently deleterious, and only rarely advantageous. Individuals carrying mutations that reduce fertility will reproduce less, and their alleles will gradually disappear from the population (known as negative or purifying selection, with a negative selection coefficient; $s < 0$). Conversely, individuals carrying mutations underlying desirable traits will reproduce more frequently, spreading their alleles in the population (positive selection; $s > 0$), which progressively adapts to the environment (Fisher, 1958). The latter represents the sole adaptive mechanism and forms the basis of any breeding scheme (Section 4).

The time required for removing or fully spreading a non-neutral allele in the population depends on its initial frequency and its selection coefficient (s), but also on its dominance coefficient (h) and on N_e. Even highly beneficial mutations may be lost forever if N_e is small and genetic drift is high. The degree of dominance also determines the phenotypic effect of an allele in heterozygous state and can range from recessive (no effect on fitness; $h = 0$), additive (intermediate effect on fitness; $h = 0.5$), to dominant (full effect on fitness; $h = 1$). Individuals with a heterozygous genotype may also exhibit superior or inferior fitness than their homozygous counterparts due to overdominance ($h > 1$) or under-dominance ($h < 0$). As a mechanism of balancing selection, overdominance can maintain both alleles segregating in the population, making trait improvement through selective pure breeding less efficient. Maintaining diversity is however beneficial in fluctuating and unpredictable environments, e.g. with varying predator and pathogen pressure (Croze *et al.*, 2016). Except for balancing selection, selection leads to a loss of genetic diversity on the loci under selection or even in a wider region. A fundamental consideration when designing breeding schemes is that dominance and selection coefficients are not independent but rather negatively correlated: since harmful mutations are often recessive, consanguinity usually entails deleterious effects, the so-called inbreeding depression (Charlesworth and Charlesworth, 1999; Hedrick and Garcia-Dorado, 2016), see Section 4: Maintaining genetic diversity. Therefore, by selecting a small proportion of individuals with desirable traits for contributing to the next generation, breeders inevitably add a drift component as well, with possibly unwanted fitness and performance effects on their farmed stock.

Overall, natural and artificial selection are powerful, context-dependent evolutionary forces. Yet, in real pop-

ulations, selection tends to be a complex process featuring interplays between selection regimes (e.g. Hill and Robertson, 1966) and genetic architectures of traits (Section 4). Multiple alleles with variable effects may segregate at any given locus, traits are frequently governed by multiple loci which may epistatically interact, and can be correlated with other traits, that may themselves be subject to context-dependent selection.

Geographical and evolutionary genetics: allele frequency changes in space and time

Population differentiation and its causes

Individual and combined evolutionary forces may act differently on populations distributed across space and time, both including single or multiple traits, and may ultimately homogenise or subdivide populations at different hierarchical levels.

Founder effects are common during colonisation of new habitats or recolonisation after population contraction (Pannell and Charlesworth, 2000; Szűcs *et al.*, 2017). Such processes usually involve comparatively small subsamples, not necessarily representative of the original population, and can hence translate into significant genetic drift or a genetic bottleneck characterised by a severely reduced overall number of alleles (Kanaka *et al.*, 2023). The same applies to the establishment of a captive laboratory or production population from one or multiple existing production or laboratory strains (Cai *et al.*, 2024; Kaya *et al.*, 2021), or from a wild population (Ewusie *et al.*, 2019; Rhode *et al.*, 2020). Extended gene flow between different natural or managed populations may result in a unilateral increase of diversity, mutual genetic homogenisation, or assimilation of one population by another, whereas inbreeding coupled with negligible migration likely increases differentiation among subpopulations (Simões *et al.*, 2010). Adaptive processes via natural selection can counteract or reinforce the effects of migration or drift.

Regardless of any selective forces, in large natural populations, genetic differentiation can also result from unequal mating probability between individuals as a function of geographic distance, i.e. isolation-by-distance (Aguillon *et al.*, 2017; Suárez *et al.*, 2022). However, isolation-by-distance patterns are sensitive to sampling design (Meirmans, 2015) and may entail a phenomenon called 'rare-allele-surfing' at the colonisation front as a consequence of comparatively rapid population expansions (Excoffier and Ray, 2008), as seems the case for BSF non-native range expansions (Kaya *et al.*, 2021).

Possibly "unexpected" structure may also reflect a Wahlund effect (De Meeûs *et al.*, 2007): genotypes drawn from a sample of individuals which are effectively not in mating equilibrium will collectively exhibit heterozygote deficiency (Hartl, 2020). Detection thereof may indicate an unnoticed sampling artefact, e.g. when field sampling captured seasonally non-overlapping generations at a given site, which can include sampling adult and immature insects at the same time (Section 2). Alternatively, genetic substructure in sympatry may reveal previously unrecognised ecological associations and/or phylogenetically derived cryptic taxa (Castillo and Barbash, 2017; Hagberg *et al.*, 2022; Sánchez-Guillén *et al.*, 2016).

Indication for subpopulation structure in supposedly standardised farming conditions can have serious consequences (see Section 4: Monitoring and managing genetic diversity). For example, widely traded BSF populations of mixed North American and Asian origin exhibit deviations from genetic equilibrium regardless of geographical area they were sourced from (Kaya *et al.*, 2021). This suggests poorly understood pre- and/or post-mating mechanisms influencing reproductive success and particularly sexual conflicts (Tomberlin *et al.*, 2025) between distinct evolutionary lineages (Generalovic *et al.*, 2023). Management itself may induce substructure over time, e.g. through discrete restocking of breeding parents where restricted geneflow between successive cycles possibly reinforces divergence, which can hamper breeding progress if undetected (Section 4).

Detecting (meta)population (sub)structure

Deciphering allele frequency variation in space and time is not only informative to reconstruct past influences on present (meta)population structure (Bradburd and Ralph, 2019). Exploring substructure helps to disentangle population relationships and inform both future monitoring of wild populations and managing breeding populations, yet requires comprehensive sampling of wild and captive populations from the entire, possibly global range. Utilising multiple complementary methods for combined analysis of metapopulation structure is generally recommended.

The above-mentioned F statistics are routinely used to characterise subdivision at different hierarchical levels of population structure. By accounting for allele frequency differences, the fixation index F_{ST} (Wright, 1965) is widely used to quantify pairwise genetic differentiation between populations, and the overall extent of partitioning due to population structure within metapopulations (De Meeûs *et al.*, 2007; Kanaka *et al.*, 2023;

Meirmans and Hedrick, 2011). Its values range from 0 meaning complete panmixia and free interbreeding, to 1 meaning complete differentiation and no alleles shared between subpopulations. Extensive matrices of pairwise F_{ST} can be visualised via heat-maps (Eleftheriou *et al.*, 2022b) or by constructing unrooted genetic dendrograms using e.g. unweighted pair group method with arithmetic mean or neighbour-joining approaches (Supplementary material, Table 1A).

Another marker-independent approach for a better general overview of inter-population comparison is Analyses of Molecular Variance, AMOVA (Excoffier *et al.*, 1992; Kanaka *et al.*, 2023). AMOVA partitions the genetic variance into proportions explained by within and among components of individuals and populations in a non-hierarchical manner, as well as by levels of one or multiple hierarchical (nested) factors, such as geographic origin or captive versus wild-sourced, as relevant for a given insect study. The extent to which pairwise F_{ST} reflect isolation-by-distance patterns among populations can be tested by correlating matrices of genetic and geographic distances using a Mantel test, usually upon adequate data linearization/transformation (Rousset, 1997). Moreover, as geography in nature may be confounded with associated factors of ecological relevance (e.g. Sandrock *et al.*, 2011b), a partial Mantel test can extend the concept of AMOVA by assessing genetic differentiation between levels of a given sampling factor (e.g. habitat) while controlling for geographic distance (see Diniz-Filho *et al.*, 2013). Allele frequency relationships of individual multilocus genotypes or entire populations can be visualised in dendrograms using geometric approaches (Cavalli-Sforza and Edwards, 1967; De Meeûs *et al.*, 2007). Alternatively, individuals or groups defined according to sampling populations, geography, captivity status, or modelled genetic clusters (see Section 3: Reconstructing demographic history) can be illustrated along the most explanatory dimensions of a multivariate space using principal component analysis (PCA) (Abegaz *et al.*, 2019; De Meeûs *et al.*, 2007; François *et al.*, 2010; Kanaka *et al.*, 2023), or via derived algorithms as implemented in discriminant analysis of principal components (DAPC) (Thia, 2023).

Evaluating population structure in farmed insects is best accomplished through a combination of population genetic indices, statistical procedures, and visual approaches. For example, geometric visualisation is principally blind to diversity components behind genetic structures. Complementary inspection of allelic richness, e.g. via mixed models (Kaya *et al.*, 2021), can then help identifying diversity hot spots.

Genetic clustering, admixture and introgressive hybridisation

Metapopulation genetic structure can further be inferred through clustering-based methods. This is particularly helpful for large datasets with numerous populations, e.g. sampled across a species' distribution range, or for allocating previously uncharacterised populations in the context of published genotypic data. The ancestry of each individual genotype is modelled as descending from a pre-defined number of potential genetic clusters, K. Ancestry coefficients can reveal pure assignment or admixture between different clusters.

Multiple implementations of model-based clustering exist (Supplementary material, Table 1A), essentially differing in their statistical procedures to find the optimal solution, conditioned on K. Virtually all implementations provide visualising relative cluster contributions to individuals within populations or geographic origin (e.g. Zhang *et al.*, 2009). Bayesian modelling using Markov Chain Monte Carlo (MCMC) techniques are popular for estimating distinct clusters from multilocus genotype frequencies, as properly accounting for the uncertainty associated with limited data (Pritchard *et al.*, 2000). With full genome sequences, maximum likelihood (ML) approaches provide much faster optimisation procedures, at limited costs in accuracy (Alexander *et al.*, 2009). More recent developments allowed to further speed up computations, including mathematical derivatives for safer and faster ML optimisation (Beugin *et al.*, 2018; Cheng *et al.*, 2021).

Caution is advised when inferring distinct clusters, because numbers of K inflated beyond biological meaning result in overfitted models and misleading estimates of admixture. It is thus crucial to refine model choice based on respective model log-likelihoods (Evanno *et al.*, 2005), cross-validation, or information criteria (Akogul and Erisoglu, 2016). This is important for both, accepting panmixia with modest isolation-by-distance across larges scales, as found in house flies (Bahrndorff *et al.*, 2020) or aphids (Sandrock *et al.*, 2011a), but also for appropriate inference of complex population structure resulting from dynamic evolutionary history (Kaya *et al.*, 2021; Nunez *et al.*, 2024). Moreover, all model-based implementations assume that population genetic principles, such as HWE, are met. Consequently, despite some robustness to violations (Falush *et al.*, 2003; Meirmans, 2012), they tend to assign unique genetic clusters to strongly diverged populations, although these

may have originated from ancient admixture events. Likewise, recent bottlenecks may inflate natural population structure (Chapuis *et al.*, 2014) and challenge traceability of heavily drifted production strains to a common yet unsampled source. Unknown demographic history, ecological associations, and insufficiently representative sampling (or extinct missing link populations) bear some risk of misleading conclusions (Lawson *et al.*, 2018; Meirmans, 2015), especially when analysing intensively managed populations and farmed stocks. Alternative models, insensitive to inbreeding or time-differences between samples, have been developed, based on factor analysis (François and Jay, 2020) or on the so-called f4-statistics, although the latter is computationally feasible up to a few hundred genomes only (Librado and Orlando, 2022). Genetic cluster analyses may thus be used as explanatory rather than a diagnostic tool, whereby inspecting results along a range of K itself can provide insights into population stratification and help scaling relative impacts in space and time (Gilbert, 2016; Gilbert *et al.*, 2012).

Explicitly describing the population history behind clustering results involves co-inferring population splits alongside historical episodes of gene flow. These population histories are often summarised as admixture graphs (Nielsen *et al.*, 2023), or inferred through coalescent modelling (Kamm *et al.*, 2020). Monitoring ongoing geneflow between production strains or with local wild populations is in fact crucial to mitigate unintended introgression in either direction, possibly challenging breeding progress of farmed strains or genetic integrity of wild populations (see Section 4: Monitoring and managing genetic diversity). Reconstructing historic and recent admixture is particularly interesting because human traffic and trade likely facilitated secondary contact between genetically distant populations, or their (repeated) translocation into uncolonised yet suitable ranges, from where further dispersal and naturalisation around the globe was possibly initiated even before the advent of modern farming of insects for food and feed. Comparable to population genetic patterns in invasive pest insects (Blumenfeld *et al.*, 2021; Sethuraman *et al.*, 2020), the global metapopulation structure of the BSF reflects decisive historic admixture events, which fuelled large scale dispersal including colonisation of multiple non-native continents through secondary and tertiary admixture (Kaya *et al.*, 2021).

To assess whether introgressed genetic material provides a selective advantage (see Section 3: Signatures of selection), as documented in multiple instances (Clarkson *et al.*, 2014; Moest *et al.*, 2020; Svedberg *et al.*, 2021), tracing its ancestral origin and fate is key. Common methods to pinpoint introgressed tracts involves variations of the Pattersons D statistic, or ABBA BABA test (Durand *et al.*, 2011; Green *et al.*, 2010). This includes the f_{d} (Martin *et al.*, 2014), and f-branch (f_{b}) statistics (Malinsky *et al.*, 2018), the latter of which can be used with a known phylogeny to identify which populations were involved in the hybridisation event (donor or receiver). Using the f-branch statistics, up to 15.2% gene flow has been detected from wild to captive populations of BSF, highlighting potentially useful strategies for maintaining diversity in closed populations (Generalovic *et al.*, 2023).

High congruent support was found for admixture in certain BSF production strains as well as for admixture between wild BSF populations e.g. from Australia and Central Europe, when comparing microsatellite data (Kaya *et al.*, 2021) and applying f-branch methods to genome-wide SNPs (Generalovic *et al.*, 2023). This highlights the possibility for efficient complementary screening strategies, where basic qualitative evidence for admixture could precede more costly measures to quantify proportion of gene sharing and identify loci involved. In-depth genomic characterisations of relative contributions to an admixed population can be relevant to inform population management.

Reconstructing demographic history

Disentangling ancient population structure from relatively contemporary population genetic dynamics (primarily reflecting anthropogenic influences) and delineating native as well as non-native ranges is challenging. Classifying diversity patterns, such as indigenous genetic hot spots vs secondary admixture, is important, but calls for inferring relative ranking of possibly confounding spatial and temporal effects. Exhaustive sampling of wild and captive populations from the entire distribution range increases the chance to infer and date a given species' demographic trajectories appropriately, including delimitation of possible domestication centres. The emerging discipline of museomics, involving DNA sequencing from historical specimens housed in entomological collections, also opens a window into the genetic past and helps in reconstructing population structure before the more recent advent of insect farming (Wandeler *et al.*, 2007). A versatile tool for comparing models of competing demographic scenarios is Approximate Bayesian Computation (ABC) (Beaumont, 2010; Csilléry *et al.*, 2010). When deep evolutionary history remains uncertain, possibly due to strong masking by contemporary human-mediated signals, formulating

and statistically assessing models based on plausible biological hypotheses is a critical step. Careful construction plus correct specification and evaluation of all reasonable demographic hypotheses, including underlying parameters and relevant summary statistics, is advised. ABC analyses have been used for microsatellite data to infer native range expansions of the BSF in the Americas, as well as its colonisation origins and courses on different non-native continents (Kaya *et al.*, 2021). To account for increasingly complex genomic data sets (e.g. Blumenfeld *et al.*, 2021), machine learning tools have already (Pudlo *et al.*, 2016) and continue to strongly improve computational efficiency for demographic history inference (Bourgeois and Warren, 2021; Fortes-Lima *et al.*, 2021; Tran *et al.*, 2024), including edible insects (Chapuis *et al.*, 2020). Recently advanced algorithm efficiency led to the development of software for inferring Ancestral Recombination Graph (ARG) that encodes the complete genealogical history of samples (Supplementary material, Table 1A). The inferred ARG is represented with a sequence of trees, where each tree represents the genealogical history of a part of the genome that was broken up by recombination. The tree sequence represents an evolutionary encoding of the data that compresses the file and enables efficient computation of relevant statistics.

Mito-nuclear phylogeographic patterns

Complementing nuclear-genetic biogeographic patterns with phylogenetically informative mitochondrial markers adds valuable insights (Edwards *et al.*, 2022), see Leite *et al.* (2014) for an insect example. Geographic associations of genealogical lineages can shed light on evolutionary cradles, vicariance effects, processes like secondary contact or directionality of range expansions. Compared to diploid nuclear loci, mitochondrial haplotypes are maternally inherited and more prone to demographic stochasticity. Accordingly, phylogeographic patterns based on mtDNA alone may not be overinterpreted. However, comparing them to demographic inferences based on nuclear markers can itself represent a highly informative evaluation for reconstructing evolutionary fates. Conclusions drawn from different marker systems may both be congruent with a certain hypothesis (e.g. diversity hot spots and dispersal routes), complexity may increase beyond individual marker perspectives (e.g. relative contributions to admixture), or a seemingly plausible narrative may be challenged by conflicting signals. For instance, nuclear genetic structure among populations of the migratory locust is substantially lower among subpopulations in areas with frequent outbreaks, despite similar levels of diversity and effective population sizes (Chapuis *et al.*, 2009). However, despite its eponymous mobility, its native range is phylogeographically highly structured. Dispersal from African hotspots established a Southern and a Northern major lineage across Eurasia and Australia that each comprise two distinct yet shallower latitudinally structured mitochondrial clades (Ma *et al.*, 2012). Further, the stronger mitochondrial than nuclear genetic structure of house fly populations in their non-native ranges (Krafsur *et al.*, 2005) likely reflects that originally colonising maternal lineages prevailed despite ongoing nuclear geneflow. The intriguing case of the BSF highlights the potential relevance of global phylogeographic data for breeding strategies. After deducting mitochondrial patterns mediated by historic translocations due to human commensalism as well as current trade for farming worldwide, reconciling native phylogeographic structure (Generalovic *et al.*, 2023; Guilliet *et al.*, 2022; Ståhls *et al.*, 2020) points at two major mtDNA clades from South and North America, respectively, separated by up to 5% COI divergence. Apart from experimental disproof of reproductive isolation of both evolutionary lineages (Ståhls *et al.*, 2020), admixture between them was decisive to initiate global non-native naturalisations (Kaya *et al.*, 2021). However, the phylogenetically older dating of the North American mitochondrial lineage (Guilliet *et al.*, 2022) appears to contrast northwards declining nuclear genetic diversity across the Americas (Kaya *et al.*, 2021). Recent genomic evidence (Generalovic *et al.*, 2023) corroborates deep phylogenetic signals similarly reflected in both mitochondrial and nuclear genomes and moreover identifies a Central American zone of mito-nuclear discordance (e.g. Toews and Brelsford, 2012). Hypothesising the BSF represents a cryptic species complex shaped by ancient hybridisation might have profound consequences on interpreting domestication history and optimising future breeding.

Signatures of selection

Population structure reflects cause and consequence of the interplay of ecological and evolutionary processes shaped by population genetics and demography, adaptive and non-adaptive forces, and their interactions (Lowe *et al.*, 2017). Differentiating between the effects of demography and selection on genomic signals is notoriously difficult (Bourgeois and Warren, 2021; Li *et al.*, 2012). Signals of selection can be confounded by genetic drift (Jensen *et al.*, 2005), most notably through small founding populations, as random fluctuations of allele

frequencies can lead to loss of genetic diversity genome wide. Founder effects can generate a strong genetic bottleneck that shows randomised signals of reduced genetic diversity, somewhat similar to that of selective sweeps (Nielsen, 2005). Adequate modelling of population demography can go a long way towards distinguishing between signals of selection and drift. Statistical methods for detecting intra-population selection, such as sweeps, may use the Site Frequency Spectrum (SFS), Linkage Disequilibrium (LD), and measures of genetic diversity (π). Cross-population selection scans similarly rely on single site markers or haplotype information (Supplementary material, Table 1A).

Hard selective sweeps are more easily identifiable (Saravanan *et al.*, 2020), and occur when a rare beneficial mutation rapidly increases in frequency via selection due to an increase in fitness (Nielsen *et al.*, 2005). Regardless of the reason, e.g. local adaptation in heterogenous environments (Tiffin and Ross-Ibarra, 2014), transfer to captivity (Montgomery *et al.*, 2010; Rhode *et al.*, 2020) or selective breeding (Hull *et al.*, 2024), this process causes surrounding linked alleles to "sweep" along with the beneficial allele by a process called genetic hitchhiking. This reduces genetic variation in the flanking regions and generates a pronounced signal of large homozygous regions in linkage disequilibrium (Smith and Haigh, 1974). In contrast, soft selective sweeps arise from standing genetic variation, which becomes selected due to sudden environmental changes, e.g. in feedstock (Hermisson and Pennings, 2005). Fixation of selected alleles can be reached but segregating on different haplotype backgrounds around the selected site. This does not produce a pronounced reduction in diversity and limits our potential to identify soft sweeps, despite they may represent the prevalent mode of adaptation (Saravanan *et al.*, 2020). Common methods of selective sweep analysis include the Composite Likelihood Ratio (CLR) test that compares the neutral demographic model for SFS with a selective model that considers a selective sweep in each genomic window. This method identified regions with sweep-like patterns associated with honeybee adaptation (Ji *et al.*, 2020) and BSF domestication (Generalovic *et al.*, 2023). Furthermore, selective sweep analysis has resolved loci involved in butterfly wing patterning, size and shape (Moest *et al.*, 2020; Montejo-Kovacevich *et al.*, 2021), and insecticide resistance (Barnes *et al.*, 2017). Under domestication, for example, up to 3% of the gene repertoire of the silkworm is impacted by selection through sweeps (Tong *et al.*, 2022).

In addition to selective sweeps, we discuss several key methods for detecting selection as outlined in Supplementary material and reviewed in Saravanan *et al.* (2020). As sweeps reduce genetic diversity locally, around selected alleles, general measures of genetic diversity are based on nucleotide diversity (π). Motivated by the same rationale, another common method to identify more recent selection signatures is to search for Runs of Homozygosity (ROH), continuous homozygous regions of the genome typically classified into short (<1 Mb), intermediate (1-5 Mb), and long (>5 Mb) runs (Peripolli *et al.*, 2017). Short runs are products of shared ancestry broken down by recombination whereas long ROH can be used to identify patterns of recent inbreeding or selection, as exemplified for BSF (Generalovic *et al.*, 2021), honeybees (Gmel *et al.*, 2023), and aquaculture species (Paul *et al.*, 2022). LD-based methods provide additional power in deciphering between drift and selection with selective signals providing a more structured pattern of linkage decay surrounding the beneficial allele whereas drift generates a more widespread non-specific pattern of LD. Methods as the integrated Haplotype Score (iHS) (Voight *et al.*, 2006) are more robust to demographic artefacts and can be combined with SFS-based methods where possible (Hull *et al.*, 2024). For inter-population detection of selection, both single-site differentiation using F_{ST} has been previously outlined above and discussed further in Section 4. Alternatively, haplotype-based methods can be used to cross compare populations for genomic regions that reached intermediate-to-high frequencies as blocks of extended haplotype homozygosity (XP-EHH), a signal that captures population-specific selective sweeps (Cai *et al.*, 2024; Montero-Mendieta *et al.*, 2019).

Further, confounding effects of sample size, N_e, and population history (including time since domestication or selection) should be considered when performing selection analyses. It is also important to note that products of selection may arise due to influences from alternative evolutionary forces such as hybridisation and adaptive introgression (Calfee *et al.*, 2020). Complementing several methods to detect selection with phenotypic analysis, e.g. GWAS or QTL (Section 4), can provide strong evidence for candidate casual genes under selection linked with the association of specific traits of interest (Hayes *et al.*, 2008; Szmatoła *et al.*, 2016). However, these metrics are still unknown for the main species of insects as food and feed despite available genomic resources.

Lastly, structural variants have significant potential to influence phenotypic differences across populations

or strains by locally suppressing recombination. What may appear as regions of the genome diverging due to selection could, in fact, be influenced or driven by structural constraints. In this context, the vinegar fly is a prime example of how substantially structural genomic variation can shape adaptation processes (Kapun *et al.*, 2023), which could be relevant in farming contexts, as known from conventional livestock (Liang *et al.*, 2024; Yang *et al.*, 2024).

4 Quantitative genetics

Most traits of importance for production and fitness (e.g. crude weight, growth rates, nutrient composition, feed conversion efficiency, egg clutch sizes, etc.) are multifactorial and complex quantitative traits controlled by multiple genes, the environment, and interactions between genotype and environment. Hence, phenotypic values of quantitative traits are often continuous, and follow a normal distribution with a specific mean and variance. This variance can be exploited by selecting and mating the best-performing individuals with each other, thereby shifting the population mean in a desirable direction over successive generations. Which traits are relevant is determined by the requirements from production systems and society. A breeding programme aims at genetically improving traits of interest through breeding, preferably avoiding negative effects on other important fitness or production traits at the population level. The expected response to selection (R) is represented by the breeder's equation; $R = h^2S$, which depends on the heritability of the trait (h^2) (see Section 4: Estimating genetic parameters), and the difference between the mean of the selected individuals and mean of the population (selection differential, S) (Figure 3) (Hansen *et al.*, 2024b). These parameters are dependent on the amount of genetic variation present in the population, the accuracy of breeding values (estimated genetic potential of individual), and the selection intensity. In this section, we discuss the main parts required to set up a breeding programme to exploit genetic variation in quantitative traits for selection in production insects.

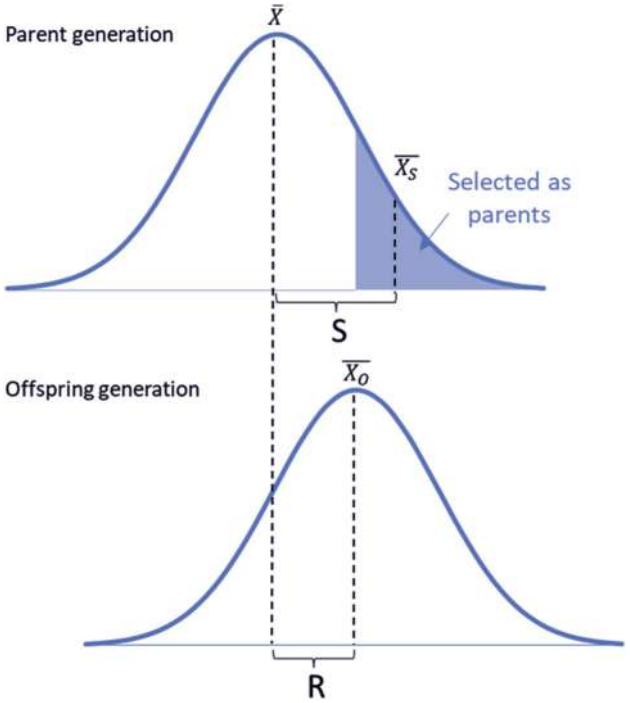

FIGURE 3 Illustration of the change in mean ($\overline{X}_O$) representing the expected response to selection (R) in the offspring generation. The difference between the mean of the individuals selected as parents ($\overline{X}_S$) for the next generation and mean of the parent generation ($\overline{X}$)) represents the selection differential (S).

Breeding objectives

To construct and implement a sustainable breeding programme, some practical considerations include the choice or establishment of the (sub-)population to be improved (see Section 4: Monitoring and managing genetic diversity), the production system the animals will exist in, and the overarching goal of the breeding and selection activities (with all these steps being dependent on one another). Insect performance is impacted by the environment, including both external environmental factors as well as, to a larger extent, factors inherent to the production system. Insect production systems can vary hugely in size and complexity, from single-batch, single-substrate systems to systems using multiple different substrate streams across individual life stages and/or generations of the animal. Nonetheless, most production systems can be defined within the parameters of the following key components: (i) the insects themselves (including community aspects such as density, sex ratio, and the genetic makeup of the population); (ii) the physical environment; (iii) fixed resources; (iv) economics; and (v) management. Once the production system for the breeding population has been described, the next step towards sustainable genetic improvement is the clear definition of a breeding goal. Having a defined breeding goal is a firm prerequisite of any breeding programme as it allows for the inclusion of several biological traits into one expression of breeding worth and thus, enables progress in selection (Nielsen *et al.*, 2014). Due to the variable nature of insect production systems, the definition of

one central breeding goal is difficult to achieve. Breeding goals can either be descriptive with a set of ideal trait descriptions, or mathematical expressions of the ideal individual with each trait weighted based on perceived (or measured) economic value, as has been exemplified in BSF (Zaalberg *et al.*, 2024). Although breeding goals are often mixed in large livestock, farmed insects have a much higher potential for clear breeding goals entirely limited to measurable traits. This is a crucial benefit that can off-set difficulties in e.g. measuring the performance of individual insects.

Relevant traits in a breeding goal for insects for food and feed typically include fertility, reproductive traits, growth, larval and pupal developmental time, and larval and pupal survival and pupation rates. These are traits that enhance production efficiency by optimising output with minimal input, reducing time to harvest, and contributing to profitability and sustainability. The traits included in the breeding goals also depend on market requirements, where body composition traits become relevant to produce insect oil and insect meal, or even fatty acid and amino acid profiles. Furthermore, traits like disease resistance or resilience could become important to maintain healthy and productive populations in the future.

Information collection

Identification systems

A selection of target traits is defined based on the breeding goal, and relevant information on the traits need to be collected to make selection decisions. For most selection strategies, pedigree recording is an essential part of information collection. To record the pedigree, a unique individual identification system is essential, as it allows recording of information in a mass rearing environment. Dust, dyes, and mutilation marking, have been widely applied in insect studies (Hagler and Jackson, 2001), which allows tracking of individuals or groups at a given time in their life cycle. The challenge arises when an individual moults and the exoskeleton is shed along with any physical identifiers, and when holometabolous insects go through metamorphosis, complicating the tracking of individuals over an extended period. Consequently, tracking through isolated or grouped rearing has been the method of choice in insect breeding so far. Individuals can be reared individually in a small, labelled cup, which allows individual selection (see Section 4: Selection). Alternatively, groups of siblings can be reared in small containers to perform family-based selection. Isolated rearing of breeding animals can introduce differences in performance between the breeding and production population (see Section 4: Genotype-by-environment interactions) and introduces confounding between genetic and environmental influences on trait variance (see Section 4: Environmental variance).

Phenotyping

A major bottleneck in insect breeding schemes includes obtaining sufficient phenotypic records for accurate selection (e.g. Klein, 1974). The short life cycle of many production insects complicates trait recording on all individuals and evaluation within available timeframes and poses requirements for phenotyping throughput, labour, and space. Additionally, random measurement errors will lead to poor reproducibility of the phenotype, leading to less successful genetic improvement of the trait. Development of high-throughput automated phenotyping methods using computer vision, sensors, and machine learning to predict phenotypes automatically could enhance phenotyping capacity and accuracy (Nawoya *et al.*, 2024) and allow phenotyping of a sufficient number of individuals for selection in a timely manner for a range of relevant traits, such as body size (Laursen *et al.*, 2021) and protein content (Cruz-Tirado *et al.*, 2023). Moreover, sex identification is not always possible in juvenile insects, imposing requirements of isolated rearing from phenotyping until selection for mating. Promising developments in e.g. computer vision to identify sex at the larval stage in BSF (Nawoya *et al.*, 2025) could support direct selection after juvenile phenotyping.

Phenotypes can be collected on selection candidates themselves; named own performance, or can be collected on family members, such as sibs or offspring. Data collection on offspring to evaluate parent performance, so called progeny testing, requires overlapping generations, hence is not suitable for insect species with discrete generations. Data collection on sibs can easily be exploited in insects and is of additional interest for recording complex traits that require sacrificing of individuals, such as detailed body composition (Bouwman *et al.*, 2024). Besides measures on individuals, group records can be utilised to obtain information on many groups while sacrificing some within-group information (e.g. Adamaki-Sotiraki *et al.*, 2023; Scieuzo *et al.*, 2023).

Genotyping

Genotypes can be a useful information source in insect breeding (Eriksson and Picard, 2021) (Section 2). The advancement in genotyping technology has increased the availability of genomic data that can be used to

reconstruct pedigrees, correct pedigree errors, or identify previously unidentified familial relatives. Genomic information can also be used to evaluate the level of inbreeding in the population for breeding management purposes. Last, genotyping phenotyped individuals is required to establish a reference population for genomic selection (see Section 4: Selection), to detect regions in the genome associated with the trait of interest, and to determine the genetic architecture of the trait (Xia, 2020) (see Section 4: Genetic architecture of quantitative traits).

Genetic architecture of quantitative traits

To elucidate the genomic architecture of quantitative traits, loci in the genome that affect the traits can be identified. These loci are called quantitative trait loci or QTLs. Once identified, genomic markers associated with the trait of interest could be used for marker assisted selection (see Section 4: Selection criterion). In insects, the vinegar fly has been the model species to study genetic architecture (Flatt, 2020; Mackay, 2004). Medically and agriculturally important QTLs have been identified and mapped in insect pests (Behura, 2006), the silkworm (Lu *et al.*, 2004) and the honeybee (Guichard *et al.*, 2021), and extending such studies to other farmed insect species should be relatively straightforward. Identifying naturally segregating loci that affect quantitative traits can be done using QTL mapping based on linkage, genome-wide association studies (GWAS), or by evolve and resequencing studies.

QTL mapping via linkage analysis

In QTL mapping, phenotypes and marker genotypes are combined from a cross between two strains that differ with respect to one or more traits. Genomic regions between markers highly associated with the trait in the offspring of the cross can be identified as the QTL underlying these trait differences (Heckel, 2003). A linkage map of the markers needs to be available. A common method for QTL mapping in the vinegar fly is creating recombinant inbred lines (RILs) (Mackay, 2010). Such lines are created by inbreeding the second generation of a cross between two lines, which allows associating phenotypes to QTLs. The downside of QTL mapping via linkage analysis is that only sites that show allelic differences between the two parental lines can be detected (Mackay, 2001), and that the genomic regions identified are usually rather broad and require additional testing to fine map the region (Mackay, 2010). An alternative is therefore association testing using genome-wide SNPs in linkage disequilibrium with the causal loci.

Genome-wide association

In GWAS, a large population of phenotyped and genotyped individuals can be used to estimate the effect of each genotype marker on the phenotype, possibly leading to candidate causal variants or genes. For GWAS, it is important to have sufficient power to detect associations, which depends on the trait's heritability, its genetic architecture, and the sample size. The set of sampled individuals should ideally be unrelated and contain several hundreds to thousands of individuals. GWAS data is commonly analysed with linear mixed models to estimate effects of each SNP. The inclusion of a relationship matrix accounts for spurious association due to family structure or population stratification (Price *et al.*, 2010; Sahana *et al.*, 2023). Alternatively, the principal components from the genotype data can be used to correct for population structure (Price *et al.*, 2010; Sahana *et al.*, 2023). As each SNP is tested individually, a correction for multiple testing needs to be applied to avoid false positive associations (Lander and Kruglyak, 1995). For example, a Bonferroni threshold (α / #SNPs) or false discovery rate threshold (Benjamini and Hochberg, 1995; Storey and Tibshirani, 2003) can be applied. Examples of GWAS in insects can be found in Hull *et al.* (2024) and Xia (2020). With the advancement of SNP arrays for insects for food and feed GWAS will become much easier to perform.

Evolve and resequencing

In evolve and resequencing studies, the genome of (pools of) individuals from a base population is compared to the genome of (pools of) individuals from a line split off from that base population that has undergone artificial selection, to identify genomic responses. The response to selection can be observed by comparing allele frequency differences between the base population and the selected line to identify selection signatures as reviewed in Schlötterer *et al.* (2015) and Turner *et al.* (2011). Multiple replicates under the same conditions, either in the same or in opposite direction, can confirm responsive alleles related to the selection criteria (Schlötterer *et al.*, 2015; Turner *et al.*, 2011). The advancement of high-throughput sequencing techniques (Section 2) combined with reduced cost for resequencing, and the possibility to use pooled samples to identify allele frequencies of each line and its base has made this an attractive method in insect species with short generation intervals (Generalovic *et al.*, 2025b).

Genetic parameters

Efficient genetic improvement requires an understanding of how much of the phenotypic variation observed is attributable to genetic (i.e. heritability) and how much to environmental factors. This information is essential for an accurate breeding value estimation as well as for developing an efficient breeding objective. Before any systematic selective breeding can be initiated, the major variance components and genetic parameters of the traits in the breeding goal need to be estimated.

The use of linear mixed models with Restricted Maximum Likelihood (REML), correcting for all systematic effects, including all available information from lineal and collateral relatives and a residual term as random effects, allows for estimation of additive genetic (σ^2_a) and residual (σ^2_e) variance (see the next three paragraphs). The model typically includes the random animal effect (the additive genetic effect) and can be extended with additional random effects, such as those originating from common environment or maternal genetics. Other covariates can be included, such as other recorded traits to include their effect on the trait of interest, or the inbreeding coefficient to evaluate the inbreeding effect on performance (Paul *et al.*, 2022). The phenotypic records can be individual or group records, where the use of group records for genetic parameter estimation and genetic evaluation naturally has consequences for the accuracy of estimates (Hansen *et al.*, 2025; Olson *et al.*, 2006).

Additive genetic variance

In breeding, the major variance component of interest originates from differences in additive genetics between individuals in the population, as offspring will directly inherit only the additive genetic effects from their parents. Estimating the additive genetic variance requires relatedness information to build a relationship matrix. Until now, the proportion of phenotypic variance in production traits made up of additive genetic variance has been estimated in farmed insects based on parent/offspring or full/half sibling records. Examples include traits in the house cricket (Castillo, 2005; Ryder and Siva-Jothy, 2001), desert locust (Chapuis *et al.*, 2021), yellow mealworm (Morales-Ramos *et al.*, 2022; Prokkola *et al.*, 2013; Sellem *et al.*, 2024), house fly (Boatta *et al.*, 2023; Hansen *et al.*, 2024a), and BSF (Bouwman *et al.*, 2022; Generalovic *et al.*, 2025a). Molecular data (e.g. SNPs) is being increasingly used to infer the genetic relationship between individuals in a population, which in turn is used to estimate the additive genetic variance if phenotypic data is also known (Srivastava *et al.*, 2023), as exemplified in BSF (Hull *et al.*, 2024).

Indirect genetic variance

Indirect genetic effects can represent an additional source of phenotypic variation. An example is maternal genetic effects, the genetic value of the mother's genome for her offspring's phenotype. In insects, maternal genetic effects have been primarily studied in honeybees (Brascamp and Bijma, 2014), but have not yet been thoroughly evaluated in insects produced for food and feed. The estimation of maternal genetic effects requires identity information of the mother of the phenotyped offspring. When variance components are derived using full-sib families, the maternal genetic effect is confounded with additive as well as common and maternal environmental effects. Genomic instead of pedigree data can help disentangling the confoundment (Lee *et al.*, 2010). Grouped animals are also affected by social indirect genetic effects, i.e. the effect of the genetic value of an individual on phenotypes of others in the group. Social indirect genetic effects were shown to influence female choice and aggressiveness in crickets (Bailey and Zuk, 2012; Santostefano *et al.*, 2017), mating rate in the burying beetle (*Nicrophorus vespilloides*) (Carter *et al.*, 2019), and position within a social network and locomotion in the vinegar fly (Signor *et al.*, 2017; Wice and Saltz, 2023). Studying and quantifying indirect genetic effects includes recording phenotypes of individuals from genetically diverse groups, which requires a system to identify individuals from different genetic backgrounds in a group (Ellen *et al.*, 2016).

Environmental variance

Environmental effects can be major sources of phenotypic variation and need to be properly accounted for when estimating genetic parameters and breeding values. Although production insects can be reared in controlled environments, common environmental effects can cause animals reared in the same container to perform more similar. When families are reared in the same container, the common environmental effect can only be disentangled from the additive genetic effect by splitting family groups over multiple environments (i.e. containers) (Bouwman *et al.*, 2022; Hansen *et al.*, 2024a). The confounding between additive genetic and common environmental components can also be disentangled by increasing genetic connectedness between management units such as families reared in separate containers (Kuehn *et al.*, 2007; Yu *et al.*, 2017). Genetic connectedness can be increased with repetitive use of one

parent with multiple mates, or by identifying unknown relatives with either more meticulous pedigree keeping or by using genomic data retrospectively. Additionally, maternal environmental effects, defined as the non-genetic contribution of females to their offspring's phenotype (Reznick, 1991), have been shown to affect the number and size of eggs, sex ratio, developmental time, growth rate, etc., in insects (Mousseau and Dingle, 1991). To estimate the maternal environmental effect it would be necessary to mix offspring across different mothers and environments. However, for genetic improvement, the priority is to separate additive genetic and environmental components, whereas it is less critical to differentiate various sources of environmental variance from one another.

Estimating genetic parameters

(1) Heritability: The evolvability of a trait is dependent on the extent of phenotypic variance that can be explained by additive genetic variance in a given population in a particular environment, i.e. the narrow-sense heritability (h^2) of a trait (Falconer and MacKay, 1996). The heritability is estimated as $\frac{\sigma_a^2}{\sigma_a^2+\sigma_e^2}$. Fitness traits, such as fertility, clutch size, or hatchability, commonly have very low heritabilities. On the other hand, non-vital morphological traits often exhibit high heritabilities, indicating significant underlying genetic variation (Mousseau and Roff, 1987). An overview of studies reporting estimates of h^2 for a range of traits in insects farmed for food and feed can be found in (Hansen *et al.*, 2024b).

(2) Correlation: Using multivariate mixed models to obtain estimates of trait covariances, the genetic and phenotypic correlation between two or more traits can be estimated as $\frac{\mathrm{cov}(x,y)}{\sqrt{\sigma_x^2 \cdot \sigma_y^2}}$ where x and y are either phenotypic or genetic values of the two traits. The traits need to be measured on related or preferably the same individuals but could also be on half or full siblings. Correlating traits at e.g. full-sib family level, using group sums or averages as the phenotype, enables estimation of correlations without maintaining information at the individual level over time (e.g. Hansen *et al.*, 2024a). Estimates can be improved if a group record of one trait is correlated to individual records of another (Ma *et al.*, 2021), for example by maintaining individual-level information at the adult stage.

Selection

Selection criterion

Selection of the best individuals as parents of the next generation can be based on several criteria. Until now, genetic improvement in insects produced as food and feed has primarily been done by selection on phenotypic performance of an individual itself, or through environment-induced selection using experimental evolution. Another strategy is to select individuals based on estimated breeding values (EBVs) that capture only the genetic part of the phenotypic value.

Phenotypes

The simplest form of selection is selection on the observed phenotype, commonly known as mass selection or phenotypic selection. Phenotypic selection does not require any relatedness information and is thus straightforward to implement in insects (Facchini *et al.*, 2022; Ma *et al.*, 2024; Slagboom *et al.*, 2024; Song *et al.*, 2022). Using phenotypes challenges multi-trait selection, because some traits are tightly integrated through genetic mechanisms, and selection on one trait might result in undesired responses in another (correlated responses) (e.g. Armitage and Siva-Jothy, 2005; Flatt, 2020). Additionally, some trait recordings require sacrificing the individual, rendering phenotypic selection only possible when selection is on directly observable traits. Although random mating should prevent high levels of inbreeding, insects usually have large full-sib family structures, hence phenotypic selection runs the risk of increasing inbreeding as relatives are more likely to perform well and be co-selected in a given environment.

Pedigree-based breeding values

Breeding values (BVs) reflect the average performance of the progeny of an individual expressed as (twice) their deviation from the population average performance. Prediction of BVs requires phenotypic information, a relationship matrix, and estimates of variance components. BV estimation uses linear mixed models and is most commonly done using Best Linear Unbiased Prediction (BLUP) (Henderson, 1950). Estimates of the fixed effects (Best Linear Unbiased Estimates, BLUE) provide valuable knowledge about the systematic variables that are constant across individuals, such as sex, age, or treatment group, and can be used to make informed decisions on management. Simultaneously, these effects are separated from the genetic effects that are of primary interest in selective breeding. BLUPs are estimated for all random effects, which would include

the additive genetic effect (either from individuals or their parents), and other variables which are not constant for all individuals, such as common environment effects. Selection on EBVs allows multi-trait selection schemes where covariance between traits are accounted for. Furthermore, weights, either arbitrary or based on economic importance (Zaalberg *et al.*, 2024), can be placed on each trait to compute a combined EBV for a group or an individual. Maintaining information on individual phenotypes in a multigenerational pedigree poses profound challenges in insect populations. Short and discrete generations imply obtaining and maintaining phenotypic and relatedness information in large quantities and computing EBVs before selection decisions can be made, which, for some species, could be just a few days. Re-identifying selection candidates when EBVs have been computed once again circles back to the challenge of tracking insects over time. If rearing full sibs in isolated groups is feasible, family EBVs can be used as the selection criterion (e.g. Hansen *et al.*, 2025).

Genomic breeding values

Alternatively, EBVs can be predicted using genomic data, for example marker assisted selection (MAS) (Fernando and Grossman, 1989). In MAS, the selection criterion is based on identifying the genotype at markers which are in linkage disequilibrium with candidate or known causal loci (QTLs; see Section 4: Genetic architecture of quantitative traits). However, a significant limitation of MAS is the lack of comprehensive knowledge of QTLs for traits of interest, or the fact that the markers used may only account for a minimal proportion of the genetic variance associated with these traits (Collard and Mackill, 2008). A step further is predicting EBVs using high density genomic markers, called genomic BLUP (GBLUP) (Meuwissen *et al.*, 2001). In GBLUP, the relationship matrix is constructed using only genomic data. GBLUP requires a reference population of phenotyped and genotyped individuals. Having this population, one can predict EBVs of unphenotyped animals based on their genotypes with higher accuracy than in the pedigree model (Schaeffer, 2006). Accuracy of genomic prediction decreases with increasing distance between reference and target population, wherefore frequent update of the reference population is crucial to maintain high accuracy (Pszczola *et al.*, 2012), especially in insects with quick generation turnaround. The use of genotypes for EBV prediction in insects is still limited, however, there have been some attempts. Genomic prediction has been performed in honeybees (Bernstein *et al.*, 2023) and a proof-of-principle study was done in the parasitoid wasp *Nasonia vitripennis*, showing its potential and highlighting considerations for practical application of genomic prediction in insects (Xia *et al.*, 2024). Although accuracies of these predictions did not reach accuracies observed in traditional livestock, both have shown a positive effect of including genomic data in predicting EBVs for insects. Additionally, the prediction can be strengthened by the combined use of pedigree and genomic data in the so called single-step GBLUP (Aguilar *et al.*, 2010; Legarra *et al.*, 2009).

Selection scheme

The selection strategy is often chosen based on the unit for which information is acquired. Phenotypic selection (Facchini *et al.*, 2022; Morales-Ramos *et al.*, 2019) and selection on individual EBVs are fitting for scenarios where the available information is collected on individuals, or where individuals are units that can be selected independently. When information is collected on families, e.g. full-sibling groups, family-based selection is a fitting strategy. This holds especially for insects, as keeping family phenotype and pedigree information is easier than collecting individual information. Two types of family-based selection include between-family selection and within-family selection, but a combination of the two can also be applied (Hansen, 2024). Between-family selection selects the best performing families to produce the next generation. Although easy to implement, it requires starting with a large number of families (>100) as in every generation families are excluded from breeding and hence inbreeding increases rapidly. Within-family selection maintains the number of families and selects the best individuals within each family to generate the next generation.

If information can only be collected on siblings due to phenotyping being invasive or sex-specific, sib-selection is an appropriate strategy. In advanced breeding programmes, several selection methods and selection rounds can be combined, which is advantageous when the breeding goal includes an array of traits with different requirements for phenotyping (invasiveness, life-stage, sexual dimorphism). The selection criterion depends on whether relatedness information is collected (EBVs) or not (phenotypes). Multi-trait breeding can also be accomplished by selecting different lines on different single-trait breeding goals and ultimately cross them to obtain crossbred offspring which outperform the parental average (Meyermans *et al.*, 2025). Other strategies, which have not been formally investigated in

production insects, include progeny selection and optimum contribution selection.

Finally, if information includes genotypes, genomic selection can be applied. This strategy allows for selection of juveniles even before they express the phenotypes or even if they do not express the phenotype (sex-specific trait). The main benefit of genomic selection in livestock arises from the shortened generation interval. This is of limited value in insect populations that already have short generation intervals and could even complicate the recording of sufficient information before selection decisions. The main benefit of genomic selection in insects might come from identifying genetic relatedness and increasing accuracy for hard- or expensive-to-measure traits, traits with low heritability, and traits that are expressed later in the animal's life, since they only need to be measured on the reference population (Calus *et al.*, 2013), similar to the situation in poultry (Wolc *et al.*, 2016) and aquaculture (Castillo-Juárez *et al.*, 2015; Lillehammer *et al.*, 2020; Luo *et al.*, 2022). For most insects, getting sufficient DNA for genotyping is generally done by sacrificing the individual, although less invasive approaches might be applicable (Section 2, e.g. Bubnič *et al.* (2020)). If sacrificed, the individual is then no longer available as a selection candidate to produce the next generation, and if damaged its suitability as a parent could be compromised; however, its DNA could be of value to predict the genetic merit of its full siblings.

Mating design

The choice of selection scheme and production method is highly dependent on the species' mating system (monogamy, polygyny, polyandry) that supports different pedigree structures (full-sib families, maternal- or paternal half-sib families) and enables different mating designs (controlled-pair mating, familial group mating, random group mating). Recent studies have investigated the possibilities of utilising different mating designs in different production insects (Hansen *et al.*, 2024a; Hoffmann *et al.*, 2021; Laursen *et al.*, 2024) and unveiled how those might differ between wild and captive rearing environments (Jensen *et al.*, 2025). Mating designs are frequently used to not only optimise genetic improvement, but also to limit inbreeding (Slagboom *et al.*, 2024). When rearing is done in random batches, rotating batches over each other at the time of mating in every generation can be used to mitigate inbreeding. In the case of family selection, males can be rotated over different families. If a high degree of mating control is achievable, optimum contribution selection (OCS) can be employed. OCS maximises the mean breeding value in the offspring while controlling the rate of inbreeding by optimising individuals'/families' contribution and constraining the increase in mean kinship in the breeding population (Meuwissen, 1997). Another approach would be to minimise the increase in mean inbreeding of the population with a fixed constraint on the desired genetic progress (Colleau and Moureaux, 2006) which has already been applied in trout and insects. The final combination of information source, selection criterion, and selection strategy are thus dependent on the possibility to control the mating strategy and mating conditions (e.g. isolated mating pairs). As knowledge is sparse, research efforts for different farmed insects will be valuable for the development of species-specific insect breeding schemes.

Experimental evolution

Experimental evolution intends to change an organism's trait of interest by applying an environmental selective regime under controlled conditions and is particularly convenient in organisms with short generation intervals, such as insects. Adaptive evolution may occur by new mutations in individuals or by a change in the population's allele frequency. The speed and effect size of the adaptive process depends on the experimental population's initial genetic variation, the number of generations, and the strength of selection. "Experimental evolution" in a narrow sense is the adaptive response of a population under controlled laboratory or field conditions to an applied selective environment. In a broader sense, it includes more controlled forms of artificial selection based on selecting specific individuals that most strongly express the trait of interest for breeding the next generation (Kawecki *et al.*, 2012). One major advantage of having an experimental population evolving is that tracking individual phenotypes every generation is bypassed by scoring phenotypes after several generations (e.g. Gligorescu *et al.*, 2023). This also spares data collection on relatedness between individuals to construct relationship matrices. Consequently, this approach sacrifices the ability to deliberately select on specific phenotypes while accounting for trait correlations, track population inbreeding, and, in some cases, control selection intensity. Another, more recent, approach is to use whole genome sequencing to compare individuals from evolved versus control populations, which permits to identify genomic loci responding to adaption.

Important aspects when setting up experimental evolution studies include replication of starting popula-

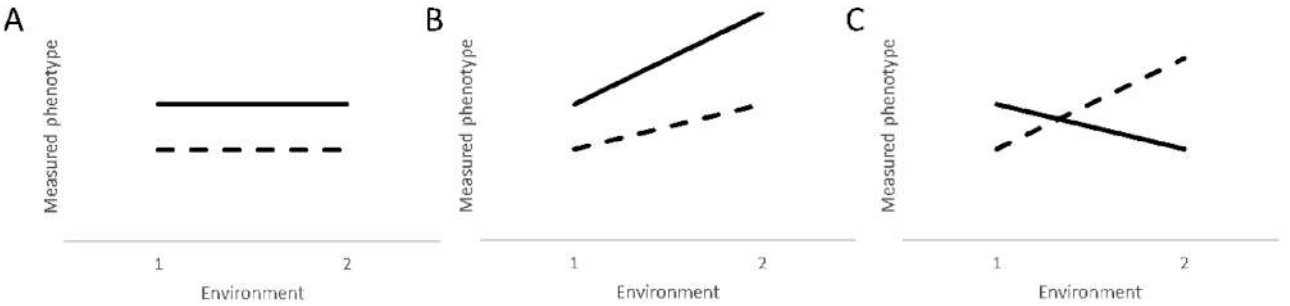

FIGURE 4 Forms of genotype-by-environment interaction, with the phenotype on the *y*-axis, environments on the *x*-axis, and the reaction norm gradient between them. Solid black and dashed lines represent different genotypes. (A) absence of G × E when all genotypes have the same response in the different environments; (B) non-crossover interaction when there is a change in the magnitude of the response without crossover (scale G × E); (C) crossover interaction when there is a reranking of genotypes in the considered environments (G × E).

tions to control for stochastic processes and possibly alternative evolutionary responses, including genetic redundancy (Generalovic *et al.*, 2025b). Control populations that are not subjected to the applied selection regime need to be set up simultaneously to control for allele frequency changes independent of the selective pressure, such as domestication effects and genetic drift (e.g. Hull *et al.*, 2024). The starting population requires sufficient standing genetic variation for the trait of interest. This can be accomplished by mixing several field-collected populations prior to the selection experiment. However, this may bear disadvantages through outbreeding depression that breaks up co-adapted gene complexes (Frankham *et al.*, 2011). Population size is another important factor, specifically the number of individuals contributing to the next generation (N_e, see Section 3: Effective population size and Section 4: Monitoring and managing genetic diversity). A final factor to consider is the number of generations. A response to selection is sometimes already visible after 5-10 generations in experimental insect populations, but it may also require longer (Lirakis and Magalhães, 2019).

An important question is whether a selected trait is maintained in commercial mass rearing conditions. Not only may the mass rearing conditions cause relaxation of selective pressure applied in the laboratory, but it may also trigger other adaptive responses that interfere with the selected trait. Due to limited studies that applied experimental evolution to insects for food and feed (but see Boatta *et al.*, 2024), information remains scarce.

Genotype-by-environment interactions

Genotype-by-environment interactions (G × E) occur when different genotypes (e.g. populations, groups of relatives) respond to environmental variation (abiotic factors, diet, microbiota, scale, etc.) in different ways, i.e. when trait expression depends on which genotype is evaluated in which environment (Figure 4) (e.g. Sandrock *et al.*, 2022). Two types of G × E can be distinguished (Falconer and MacKay, 1996): (i) crossover G × E, where genotypes can change ranking between environments (Figure 4C); (ii) non-crossover G × E, where genotypes retain ranking, but the differences are larger in one environment compared to another (Figure 4B), or a combination of both. G × E can reduce efficiency of selection when exchanging individuals across environments since it implies that genotypes differ in genetic potential to adapt to different environments (Falconer and MacKay, 1996). This is especially true for the case of reranking, where there is no single superior genotype across environments, and hence selection in one environment will lead to a lower genetic gain than expected in another. In addition, under G × E, not only mean performance of a given genotype may change depending on environment, but also respective variances, which holds equal importance for consistency in production (Laursen *et al.*, 2024).

G × E interactions have been widely demonstrated being a key driver in evolutionary ecology, with plenty of examples in entomology research (Gamboa and Watanabe, 2019; Sandrock *et al.*, 2010; Santos *et al.*, 1994), conventional livestock (e.g. Fodor *et al.*, 2023; Wackchaure *et al.*, 2016) and aquaculture (e.g. Sae-Lim *et al.*, 2016). Well-characterised examples are known from insect models such as the honeybee (Costa *et al.*, 2012), or the vinegar fly, including a plethora of life history traits and metabolic phenotypes (Flatt, 2020; Reed *et al.*, 2010). Several available studies suggest this aspect is also relevant in insects for food and feed (Generalovic *et al.*, 2025a; Greenwood *et al.*, 2021; Laursen *et al.*, 2024; Sandrock *et al.*, 2022; Silvaraju *et al.*, 2024; Zhang *et al.*, 2024).

Two major types of models are used to study G × E interactions: multi trait model (MTM) and reaction

norm model (RNM). The multi trait model is mostly used when few categorically classified environments are studied. The phenotypic performance of the studied genotype recorded in different environments are considered as a separate, but potentially correlated traits (Falconer, 1952). The reaction norm model uses a continuous variable to describe environments that allows to classify genotypes in this gradient and to identify environmental factors causing G × E (Hayes *et al.*, 2016; Sae-Lim *et al.*, 2016).

Awareness of the presence and the extent of G × E is key, for ensuring selection response. If not accounted for, performance and reproductive fitness cannot be predicted for any production setting that differs from the selection regime of the nucleus population (Mulder and Bijma, 2005). In insects, the main challenge are thus environmental differences within the nucleus population compared to rearing conditions on production farms, e.g. including climate control, diet quality and provision, insect density, etc. Moreover, in evolutionary diverse taxa like the BSF where mitochondrial haplotypes comprise substantial coding differences (Guilliet *et al.*, 2022), complex interactive effects of diet and mitonuclear epistatis may be involved, as shown in the vinegar fly (Mossman *et al.*, 2016).

Monitoring and managing genetic diversity

Establishing a breeding population

Insect species farmed for food and feed generally lack a long history of domestication or closed captive rearing, with few exceptions. As a result, well-characterised breeds, strains, or lines that are specifically adapted or bred for particular purposes are not readily available and must be established. The establishment of a breeding population begins with the careful selection of founder individuals that can be (i) initiated from an already commercially farmed population; (ii) collected from wild populations; or (iii) created from a combination of wild and/or captive origins; and it continues with a stringent monitoring of its population genetic structure. Establishing a large breeding population that encompasses high genetic variation is more likely to capture diversity that can be leveraged in breeding while simultaneously reducing the risk of stochastic loss of genetic variants (Frankham, 2005). Conversely, reproductive success within the founder population is equally important to prevent bottlenecks and resulting founder effects. To mitigate this risk, it is essential to avoid assortative mating among founders, which does not seem to be relevant in genetically uniform populations (Laudani *et al.*, 2024) yet appears to become relevant when crossbreeding substantially differentiated subpopulations (Hoffmann *et al.*, 2021; Kaya *et al.*, 2021). Despite an absence of general recommendations regarding the census and effective population size of farmed insect populations, guidelines for effective population size in livestock generally suggest a low range of 50-100 (Woolliams *et al.*, 1998). To maintain the evolutionary potential, an effective population size of >5000 could be needed (Franklin and Frankham, 1998) which would entail a census population size above this number.

Maintaining genetic diversity

Long-term genetic improvement depends not only on the establishment of diverse populations, but equally on maintaining genetic variation to improve populations' robustness and production. Captive, isolated populations are at risk of genetic erosion due to average relationship between individuals increasing every generation. The life-history characteristics of many insect species, including short life cycles, high fecundity, high juvenile mortalities, large variances in family size, and skewed progeny contributions, renders them particularly susceptible to drastic fluctuations in population size. This, combined with inbreeding, results in an increase in homozygosity that reduces genetic variation over generations. Increased inbreeding can lead to inbreeding depression, decreasing the phenotypic value for fitness and possibly other economically important traits (Hedrick and Garcia-Dorado, 2016; Leung *et al.*, 2025; Woodworth *et al.*, 2002). Increasing homozygosity further causes the loss of variability for currently neutral traits that can become economically important in the future (Meuwissen *et al.*, 2020). Primary strategies for maintaining genetic diversity beyond establishing a diverse breeding population and ensuring high effective population size entail: (i) Managing the rate of inbreeding below 1% per generation (Bentsen and Olesen, 2002) through careful mate choice decisions that minimises mating between relatives. Such a strategy requires pedigree records or use of molecular markers (see Section 4: Information collection); and (ii) Population augmentation, which is the introduction of unrelated individuals to a population to restore genetic diversity and counteract the effects of inbreeding depression through outcrossing (Kronenberger *et al.*, 2018).

Little is known about genetic erosion and effects of inbreeding in captive insect populations beyond recent indication of substantial fitness reductions upon repeated sibling mating (Laudani *et al.*, 2024). Counter

to livestock breeding experience, insects exhibit many fold the generational turnover, resulting in rapid loss of genetic variation (Rhode *et al.*, 2020). Conversely, insects might tolerate higher levels of inbreeding than livestock (Cai *et al.*, 2022; Swindell and Bouzat, 2006). Purging of recessive deleterious alleles through inbreeding could mitigate negative effects of inbreeding depression (Caballero *et al.*, 2017; Pérez-Pereira *et al.*, 2021). However, such a management strategy comes with a risk of population collapse and is not guaranteed to be efficient in small populations (Frankham, 2005). Inbreeding depression can even be environmentally dependent, wherefore purging of deleterious recessives might only be effective in specific environments (Bijlsma *et al.*, 1999). The general recommendation is to avoid inbreeding and maintain high levels of genetic variation through management practices and continuous monitoring and evaluation of the breeding population.

Outbreeding depression

Introducing new genetic material to a breeding population is highlighted both as a breeding strategy (crossbreeding) and as an inbreeding management tool (population augmentation). Such strategies can be beneficial but must be used with caution. Although they can positively impact phenotypes through hybrid vigour and introduction of new genetic variation, they may also result in the loss of genetic gains achieved through selective breeding for production traits. Detrimental loss of fitness in the population due to outbreeding depression may also be an outcome (Kurbalija *et al.*, 2010; Peer and Taborsky, 2005), likely due to disruption of co-adapted gene complexes and/or genomic incompatibilities manifesting in the first or later generations (Edmands and Timmerman, 2003). Intentionally seeking heterosis to enhance fitness or production traits may be highly sensitive to the parental counterparts involved in interbreeding. In some insect species, outbreeding can yield beneficial effects (Singh *et al.*, 2002; Szűcs *et al.*, 2017), while in others, it may have no significant impact (Fountain *et al.*, 2015; Leung *et al.*, 2025). Complex metapopulation structure with highly diverged evolutionary lineages or even unrecognised cryptic taxa (Generalovic *et al.*, 2023; Hagberg *et al.*, 2022) may indicate possible risks of outbreeding depression. Yet, prediction thereof solely based on genetic distance is difficult. While neutral genetic drift can have strong impact on genome-wide differentiation (Cai *et al.*, 2024), the segregation of non-recombining structural genomic variations was indeed shown to mediate and maintain insect ecotype differentiation through environmental adaptation (Kapun *et al.*, 2023). Hence, the effect, whether positive or negative, on desired traits or fitness when employing crossbreeding is likely source and context dependent. Consequently, unmonitored interbreeding intended to randomly increase genetic diversity may lead to counterintuitive unwanted consequences for fitness in the short term and ultimately affect the population's genetic makeup in the long term.

Protecting wild populations

The existence of wild alongside managed populations of several farmed insect species calls for an increased awareness for conservation genetics responsibilities. Although there are regional differences in regulating insect productions, as well as in motivations for their implementation, the insect production sector should develop strategies to safeguard the integrity of local wild populations across both indigenous and non-native ranges. This will warrant the preservation of unique genetic profiles for future breeding purposes. For the same reason, continuous monitoring of introgression from insect farms into the wild is recommended to evaluate and trace regional invasion potentials (Bang and Courchamp, 2021). Even if largely maladapted under natural selection, introgression from strains selected in mass-rearing contexts has the potential to change life history traits of wild populations possibly broadening capacities for ecological adaptation (Beaurepaire *et al.*, 2024; San Jose *et al.*, 2023). Invasiveness could significantly destabilise natural ecosystems and worsen public perception of insect farming (Lourenço *et al.*, 2022). Such unwanted developments need to be proactively prevented. Possible strategies beyond physical containment and geographical isolation include genetic monitoring of both farmed strains and wild populations, reproductive control through genetic modifications, selective breeding for low fitness in the wild, as well as development of industry guidelines to minimise escapes and ensure responsible management practices across the sector.

5 Functional genetics

Functional genetics refers to the study of the genetic architecture of organismal variation. Technological developments have facilitated the transition from targeted studies on few genetic markers to applying whole genome scans. The focus in the context of breeding insects for food and feed is to identify biological relationship between genes (and variants) and phenotypes

of interest, such as reproduction, growth, resilience, or resistance to disease. In its simplest form, variation in protein coding regions of the DNA sequences lead to variation in mRNA sequences, which are translated to the active molecules. Therefore, it is generally expected that all variation in phenotypic performance is based on segregating variants of protein coding genes, and regions that regulate their expression.

For traits with a simple genetic architecture, where single or few loci with large effect determine the phenotype, progress has been made, especially within the field of human health and disease research (Reed *et al.*, 2011; Slavkin, 2014). Generally, however, pin-pointing genes and genetic variants underlying organismal traits remains difficult. This is partly due to many traits being highly polygenic and controlled by many genes, each with a minor effect, but also since phenotypes are affected by numerous additional (environmental) factors (Hansen, 2024) (Section 4). Multiple intermediate processes and specifically interfering factors contribute to the final phenotype. Thus, the direct association between gene variant and trait value is often hard to identify and predict. The strength or direction of a genotypic effect on a phenotype often depends on the environmental condition (see Section 4: Genotype-by-environment interactions). Recent investigations of farmed insects have observed differential performance and composition of different strains based on administered diets pointing out phenotyping challenges due to G × E interactions (Generalovic *et al.*, 2025a; Gligorescu *et al.*, 2023; Greenwood *et al.*, 2021; Sandrock *et al.*, 2022).

The main mission for functional genomic studies is to (i) generate biologically meaningful interpretation of large datasets including genetic/genomic information, but also transcriptomic (gene expression) and similar large scale 'omic' data, and (ii) identify biological relationships between genetic/genomic variation and organismal (functional) phenotypic variation. Intricate knowledge of the biological system being investigated is required to achieve clear and robust interpretation, including functional annotation of genes and molecular pathways and a deep understanding of the ecology and/or requirements and conditions experienced by the studied organism (Muchina *et al.*, 2025). In the following, we discuss applications and achievable goals of available techniques and highlight common pitfalls. Illustrated by examples, we provide general recommendations how to apply functional genomic tools.

Current trends in insect functional genetics/genomics

Currently, functional genomics publications related to insect farming are heavily skewed towards two commercial species: the yellow mealworm and the Mediterranean field cricket make up over 70% of the literature pool. This is because they have long been studied as model organisms for molecular mechanisms of development, regeneration, immunity, and host-pathogen interactions, and in part, due to their comparatively easy rearing in the laboratory (Mito and Noji, 2008; Petronio Petronio *et al.*, 2022). While research on these organisms provided a solid foundation of fundamental research, there has been a shift towards more applied research aimed at understanding gene function and regulation that could advance the field of insects as food and feed. This shift is primarily driven by numerically strongly increasing literature records on the BSF (Athanassiou *et al.*, 2025; English *et al.*, 2021; Siddiqui *et al.*, 2024), fuelled by technological developments that allow functional genomic studies and molecular manipulation with little prior information (Figure 5). While high quality genomes are still much desired (Oppert *et al.*, 2023), many studies can be performed as sequencing allows the *de novo* construction of annotated genomes and transcriptomes, permitting targeted RNAi and gene editing.

Beyond the focus on progress directly on commercially farmed insects, there is a wealth of knowledge on other model species systems like red flour beetle (Campbell *et al.*, 2022; Kumar *et al.*, 2018) and vinegar fly (Mackay and Huang, 2018), which can serve as inspiration for adopting approaches to tackle yet unattempted work in the target species for food and feed.

Techniques and their applications

The first step in identifying the genetic architecture that underlies a trait of interest is a choice between investigating associations between genotype (DNA-sequence) and/or expression level (mRNA abundance) as these approaches provide different insight. Following the generation of lists of candidates, these should be interpreted via bioinformatics analysis and then functionally verified. To achieve this, it is also required that accurate phenotyping is performed to support the analysis. Ideally functional genomic studies include all these steps.

Associating genotype with phenotype

The first step is typically some form of genotype-phenotype correlation analysis (e.g. QTL mapping or GWAS, see Section 4: Genetic architecture of quantitative traits) to identify a statistical association. Yet, these

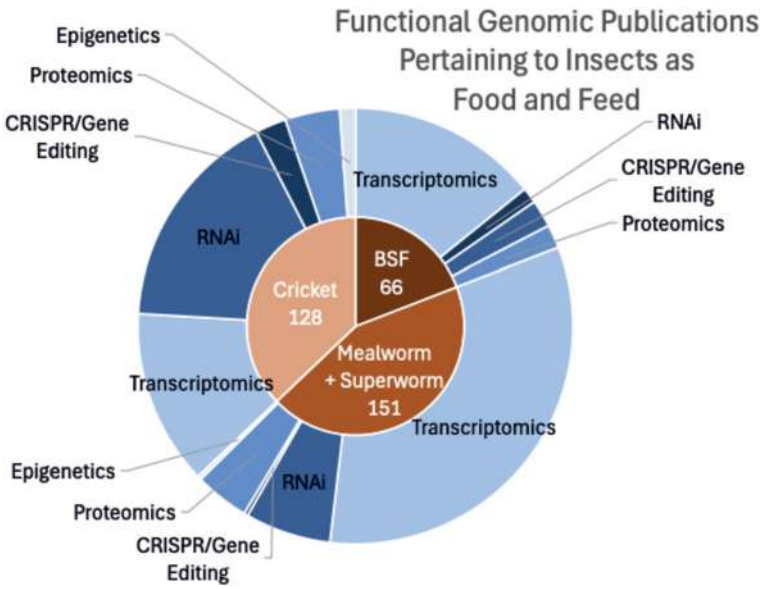

FIGURE 5 Publications (up to April 2024) related to functional genomic studies done on three groups of commercially farmed insects (data curated from Web of Science, excluding publications related to insect-fed livestock). Cricket refers to five species commonly reared for food and feed (*Brachytrupes membranaceus*, *Gryllus assimilis*, *Gryllus bimaculatus*, *Gryllotalpa orientalis* and *Acheta domesticus*).

approaches do not identify the underlying molecular mechanisms causing the observed phenotypic variation. QTL mapping studies identify large effect loci which can then be scanned for genic content. Resolution is coarse and unlikely to yield unique candidate genes and gene variants, however, promising candidates can then be further characterised through analysis of gene functions and pathways/networks. Re-sequencing these genes may further highlight DNA sequence variants that can be explored in candidate gene fine-mapping association studies (e.g. Hull *et al.*, 2024). The impact on sequence variants (e.g. SNPs and indels) can be investigated through *in silico* strategies to find, for example, if a base pair substitution leads to an alteration in amino acid sequence and peptide folding. Various *in silico* approaches have also been developed to find conserved regulatory elements (e.g. promoter region motifs and other 'enhancer' sequences in the 5′- or 3′-untranslated regions or intron splicing sites).

Gene expression analysis

Transcriptome analyses is a complementary approach to screen for genes showing expression differences associated with a phenotype, providing insights into when and where mRNA of different genes is accumulated in an organism (Singh and Verma, 2022). For example, the increase in mRNA levels of specific genes in different tissues of silkworms served to identify and describe the regulation of silk gland activity (Masuoka *et al.*, 2022; Yokoi *et al.*, 2021), while RNA sequencing (RNAseq) under stress conditions served to link Dipteran transcriptomics to phenotypes and indicate a potential role of genes responding to the stressor (de Oliveira *et al.*, 2021; Hull *et al.*, 2023; Yadav *et al.*, 2017). Similarly, transcriptional profiling in BSF has proven useful to explore chemoreception of different developmental stages (Scieuzo *et al.*, 2021) or in a sex-specific context during mating or oviposition (Xu *et al.*, 2020), the regulatory basis of nutrient, energy and particularly lipid metabolism during larval growth stages (Giannetto *et al.*, 2020; Peng *et al.*, 2023; Sukmak *et al.*, 2024; Zhu *et al.*, 2019), or specific immune responses (Moretta *et al.*, 2020; Vogel *et al.*, 2018) that possibly induce production trade-offs (Shah *et al.*, 2024), as well as gene expression differentials according to selection for increased growth (Hull *et al.*, 2023) or temperature tolerance (Ma *et al.*, 2024). The relevance of gene regulation is illustrated in the silkworm (Tong *et al.*, 2022) where large parts (55%) of the structural variation found in pan-genomes affected regulatory regions. Importantly, transcriptomes do not necessarily identify causal variants underlying a change in phenotype – for example alteration in expression of a transcription factor could affect expression levels of many downstream genes. Therefore, QTL/GWAS approaches are highly complementary to transcriptome experiments.

Measuring gene expression via transcription is one of the most accessible and widely utilised approaches

for investigating gene function and regulation, with a wide array of tools from high-throughput RNAseq of whole transcriptomes (De Wit *et al.*, 2012) to targeted techniques such as qPCR. Proteins are in most cases functional molecules and can be measured using Western blots, ELISA, and mass spectrometry-based methodology. These approaches do not always produce concordant results, in part because abundance of mRNA does not necessarily translate to abundance of proteins: abundance does not reveal much about turnover dynamics (transcription rate, translation rate, degradation rate). For example, measuring the expression of *vitellogenin*, a gene crucial for egg development and oogenesis, in the yellow mealworm in response to a molecule secreted by a parasite (Warr *et al.*, 2006) found significantly higher mRNA abundance in infected beetles compared to uninfected controls, nevertheless, associated protein levels were unexpectedly lower.

Given the rapid advancement of sequencing technologies, differential gene expression studies using whole transcriptome sequencing (via RNAseq) is the most common approach. For example, Hull *et al.* (2023) used RNAseq to investigate differential gene expression between selected and unselected lines of BSF to find genes associated with larval growth. RT-PCR can be applied to candidates to expand sampling and validate the correlation between transcription and phenotype. Measuring gene expression using RT-PCR comes with substantial technical and biological variation (as gene expression is extremely sensitive to environmental conditions). Thus, care must be taken to randomise and normalise intermediate steps in the procedures, by investigating and validating reference genes specifically for each treatment (Gao *et al.*, 2019; Rhode and Greenwood, 2023) or applying other measures to account for systematic, technical and random variance (Heckmann *et al.*, 2011).

Careful interpretation of the generated data is needed. A major challenge of linking transcriptomic data to gene function is that data can be noisy due to random interactions among genes, their products, and environmental factors. The existence of segregating gene variants might not necessarily be manifested as differential gene expression in a transcriptomic study, and differential gene expression may not be detected in studies of genetic diversity. Redundancy in genetic pathways poses another challenge, as multiple genes can compensate for each other's functions (Generalovic *et al.*, 2025b). Finally, gene variants and differential gene expression with critical effects in specialised tissues or developmental stages might easily be overlooked in whole body extractions of single stages (Oppert *et al.*, 2023; Scieuzo *et al.*, 2021).

Fully dissecting the functional impact of genotypes on phenotypes necessiates a wider look at multiple tiers of biological organisation. Integrating transcriptomic data with other 'omics' – such as proteomics, metabolomics, and epigenomic scans – helps constructing a comprehensive view of gene functions (Gallagher and Chen-Plotkin, 2018; Maji and Garg, 2013). The number of proteomics studies in BSF has increased (e.g. Bose *et al.*, 2023; Lu *et al.*, 2021; Rabani *et al.*, 2019). Other studies propose or apply a systems genomics approach with the integration of various multi-omics data (Aagaard *et al.*, 2022; Kadarmideen, 2014; Suravajhala *et al.*, 2016). Next to successful studies (Lecheta *et al.*, 2020), in some cases the integration of data across levels of biological organisation does not provide clear functional answers (Malmendal *et al.*, 2013). Enzyme concentration does not necessarily predict enzyme activity, and resulting metabolites (collectively the metabolome) is complex in function and devoid of info on the dynamics. Accordingly, the decrease of a metabolite under certain conditions can signify decreased production (less need) or increased consumption (more need). As such, no change (at any level) could cover a range of vastly different conditions with different biological consequences but is unlikely to be detected.

Linking gene expression changes directly, e.g. to a response to a stressor relative to wider mechanistic consequence of the treatment can be challenging. For example, while high fold change induction of heat shock proteins correlates well with exposure to increased temperature, causal interpretation is less obvious. Gene expression up-regulation could reflect capability to accommodate stress, or that the organism is severely stressed and barely able to withstand (Morfin *et al.*, 2023; Sørensen, 2010). Finally, though tempting, equating fold changes with biological significance is not necessarily meaningful, as modest regulation of key genes could have huge effects.

To be expressed, a coding region must be accessible for transcription. Epigenetic processes modulate gene expression, with phenotypic consequences. These modifications can be genetically controlled or induced by the environment; for instance, DNA methylation patterns in the house cricket upon exposure to graphene oxide results in multigenerational oxidative stress (Flasz *et al.*, 2023a,b). Polyurethane foam-feeding yellow mealworm results in stage-specific changes in mitochondrial DNA methylation patterns associated with decreased

ATP synthesis (Guo *et al.*, 2019). Further, leg regeneration in Mediterranean field crickets is epigenetically regulated by histone H3K27 methylation (Hamada *et al.*, 2015). Silkworms have exhibited increased DNA methylation during domestication and reduced hatchability when a DNA methyltransferase (*Dnmt1*) is knocked down suggests important adaption to managed environments (Xiang *et al.*, 2013). Epigenetic profiling studies in insects identified increased expression of epigenetic modification enzymes in the reproductive organs of domesticated silkworm populations (Gao *et al.*, 2020) and the role of epigenetic control of growth in the red flour beetle (George *et al.*, 2019). Given the supposed high relevance particularly in production insects (Mukherjee and Vilcinskas, 2019), further investigations into the epigenome of the core insects as food and feed species will develop our understanding of the high phenotypic plasticity observed (de Carvalho, 2023) that is rarely directly ascribable to DNA sequence variation (Glastad *et al.*, 2019). Epigenetics and methylation studies through whole genome bisulphite sequencing (commonly used to detect DNA modification), are still relatively scarce in insects, except for social insects (Adusumalli *et al.*, 2015; Yagound *et al.*, 2020). Generally being lower than in mammals, levels of cytosine methylation vary substantially across arthropods, and Dipterans (e.g. BSF) seem to have lost this main methylation mechanism, suggesting other mechanisms operate in this order (Bewick *et al.*, 2017). In a breeding context, to mitigate unwanted 'maternal' effects, e.g. in G × E studies, it is recommended to rear experimental populations under equal conditions for multiple generations prior to the trial (Sandrock *et al.*, 2022).

Bioinformatic prediction of function

Several bioinformatic tools can improve our understanding of candidate gene roles and assist experimental verification of gene functions. Functional interpretation of candidate genes can be restricted by poor annotations. Gene functions can be predicted by comparison to genes of interest with known sequences in databases (e.g. BLAST). Numerous specialised databases are available to support insect genomics research. For example, the Insect Cytochrome P450 Database contains 66 513 P450 genes from more than 680 insect species serving as a comprehensive resource for predicting gene function and aiding to understand their evolution in insects (Wu *et al.*, 2024). Other resources include the CAZy (Cantarel *et al.*, 2009) and iCAZyGFADB databases which integrate multiple annotation tools for genomic and transcriptomic data to identify carbohydrate active enzymes (Fu and Yang, 2023), the InsectBase 2.0 which provides data on more than 800 insect genome and 25 000 transcriptomes covering more than a million of annotated genes (Mei *et al.*, 2021), and the AlphaFold prediction tool for protein structure and function (Jumper *et al.*, 2021). Generally, the integration of multiple databases can enhance accuracy of prediction (Marques de Castro *et al.*, 2022; Shim *et al.*, 2017), yet bioinformatics-based prediction has limitations: databases capture only publicly available information. Even though a lack of functional evidence in databases does not necessarily mean a computational prediction is incorrect, it necessitates further "wet" functional genomics studies to characterise gene activity and possible roles in cellular processes (Kikuchi *et al.*, 2017; Urzúa-Traslaviña *et al.*, 2021).

Functional characterisation of candidate loci

The ultimate goal is to experimentally verify the effect of a particular gene or genetic variant on function. One approach is RNAi, which exploits a naturally occurring biological process wherein small RNA molecules inhibit protein expression by neutralising targeted mRNA. Researchers can leverage this molecular machinery to knock down genes of interest and gain insight into their function (Bellés, 2010). RNAi was utilised to explore metallothionein genes in BSF larvae, potentially responsible for metabolising cadmium, to better understand mechanisms relevant for bioremediation (Zhang *et al.*, 2021). By systematically silencing three of these genes upon cadmium-exposure, knock out of one gene (*BSFMT2B*) decreased larval weight and increased mortality, suggesting its role in cadmium detoxification and tolerance.

In addition to RNAi, CRISPR/Cas9 technology has been widely used in insect functional genomics (Shirai *et al.*, 2022), including the vinegar fly (Bier *et al.*, 2018; Sun *et al.*, 2015), the fall armyworm, *Spodoptera frugiperda* (Wang *et al.*, 2024), and the Mediterranean field cricket (Matsuoka *et al.*, 2024). The technology enables targeted mutagenesis and can be used to precisely knock out a gene to understand its function (Hillary and Ceasar, 2024). It has also been used to insert a reporter gene into specific loci enabling visualisation of expression patterns (Lo and Matthews, 2023). CRISPR gene editing has been successfully carried out in BSF, yellow mealworm, and various cricket species (Bai *et al.*, 2023; Chen *et al.*, 2023; Deng *et al.*, 2023; Dossey *et al.*, 2023; Extavour *et al.*, 2019; Gunther *et al.*, 2024; Inoue *et al.*, 2023; Nakamura *et al.*, 2022; Oppert *et al.*, 2023; Sui *et al.*, 2024; Zhan *et al.*, 2020). One application of this technology, in both research and breeding

efforts, are visible marker genes, e.g. affecting pigmentation. Easily observable traits can be linked to genes of interest to track genetic modifications and breeding outcomes. To date, two genetic markers have been investigated in *BSF*: mutations in members of the yellow gene family resulting in a pale-yellow phenotype (Dong *et al.*, 2024) and heritable mutation of the white gene, commonly used as a marker in other insect models, resulting in a white-eye phenotype in larvae and adults (Sui *et al.*, 2024).

Prior to CRISPR, most genetic modification involved insertion of genetic constructs into random genomic locations. A common approach is to use the piggyBAC vector to insert a gene of interest with a promotor sequence, and commonly also a marker such as GFP. Recent work has established a system for over expression of target genes in BSF (Kou *et al.*, 2023). Various overexpression systems have been reported and include viral transduction systems such as the *Anopheles gambiae* densovirus (AgDNV), which allowed high gene expression levels in the major malaria mosquito (Suzuki *et al.*, 2014). In the vinegar fly, overexpression of targeted genes can also be achieved using the GAL4-UAS system: female flies carry the gene of interest under the UAS (Upstream Activation Sequence) promoter and are crossed with males expressing the GAL4 transcription factor. GAL4 binds specifically to the UAS which acts as a promoter only in the presence of GAL4 leading to the overexpression of the targeted gene (Brand and Perrimon, 1993; Duffy, 2002). The system does not only allow the over-expression of *D. melanoagster* genes but has also been used to express human genes in the vinegar fly model (Ma *et al.*, 2003).

Animals generally tolerate genome changes much less than plants, and there is a risk that downregulating or knocking out a gene will result in inferior performance, due to the manipulation making the organism generally weaker rather than the gene being directly related to a phenotype in question. Approaches to accommodate this problem include knock-down/knock-out of additionally randomly selected genes, screening additional non-targeted phenotypes, or showing that the phenotype can be improved by over-expression. Ultimately, compared to easier knockout experiments, using knock-ins to replace natural variants and investigate their effect on the phenotype are biologically more meaningful. Gene editing aids characterising functional causalities to inform selective breeding on causal genes or even natural variants relevant for variation in specific production traits. However, natural gene variants could also be uniquely altered or interchanged depending on genetic architectures and population genetic background, respectively. In this latter case, genetic modification can represent a form of genetic improvement, synonymous to the goal in classical breeding.

The challenge of high throughput phenotyping

Both initial QTL/GWAS screens and subsequent phenotyping of functional experiments such as gene knockouts rely on statistical associations between phenotype and genotype. One challenge is to obtain large samples of phenotyped individuals required. In insect breeding research, the generation of fast, precise, and high-throughput phenotyping is the major bottleneck in experimental and commercial workflows, in comparison to obtaining high quality genome sequence data, which is now relatively straightforward. Technical developments allowing high throughput phenotyping approaches are especially needed for quantitative traits, where numerous genes are involved and large samples are needed to identify associations with phenotypes, especially for traits that are expressed differently in different contexts (Houle *et al.*, 2010).

However, promising research in phenomics in other high density and high-throughput animal production systems, like aquaculture, might inspire solutions for insect farming (e.g. Freitas *et al.*, 2023; Fu and Yuna, 2022). High-throughput phenotyping in insect models has been demonstrated (Hansen *et al.*, 2024a; Laursen *et al.*, 2021; MacLean *et al.*, 2022). Population-level studies frequently reveal a complex array of gene-environment interactions. These interactions can differ significantly among various populations, across different developmental stages, and within distinct tissue types of the same species (Houle *et al.*, 2010). Investigating population-level variations often uncovers complex patterns where numerous genes interact with environmental factors to influence phenotypic outcomes (Wilson *et al.*, 2003). In insects, these complex interactions are further confounded by species reproductive behaviour and life history traits that make insect populations highly dynamic and often subject to chance demographic shifts (see Section 3: Evolutionary forces) over temporal and spatial scales, that can quickly alter the genetic background of a population (Hull *et al.*, 2024; Kaya *et al.*, 2021; Rhode *et al.*, 2020).

Taken together, complementary work on model insect species will be largely transferable and should inform future efforts. Nevertheless, particularly in a breeding context, awareness on presumably widespread highly population-specific responses as well as respec-

tive developmental and environmental interactions is crucial. Generation of sequences and other massive 'omics' datasets is no longer the bottleneck, while competences and resources for analyses and accurate phenotyping increasingly are.

6 Discussion

The study of genetics of insects farmed for food and feed is a recent research discipline. While it is rapidly expanding with dedicated societies, conferences, journals, and an increasing number of papers, there is still much to be learned. The development of the research area builds on well-established traditions in, on the one hand, quantitative genetics and breeding of domestic animals and, on the other hand, experimental and fundamental genetic research on model insect species. However, insects are an evolutionarily old and functionally diverse group, with major orders, such as Coleoptera (beetles) and Diptera (flies and mosquitoes), having several hundred million years of independent evolution. Two important consequences arise from this. First, insects show diverse biological characteristics that differ in many aspects from traditional livestock (e.g. cows, pigs, chicken), which often prevents direct translation of knowledge. Second, the diversity in insect reproductive systems and life-histories means that extrapolation among species demands careful verification. In this article of the BugBook, we have compiled the current knowledge of using genetics to explore evolutionary histories, analyse contextual population genetic patterns and functional impacts, and inform the management and selective breeding of farmed insects. This goal is what conceptually unites the four thematic sections on genetic research and their differentiated approaches and analyses outlined above.

Insect biology

Insects are small, have short generation times, produce numerous progenies, and are ectotherms, all of which separates them from most traditional livestock. In addition to fundamental developmental differences between holo- and hemimetabolous insects, their various natural ecological niches imply that they have evolved a wide range of adaptations to thrive, including specific behaviour and cognitive abilities. Farming management practices thus need to account for these adaptations in a welfare context. The diverse biological features of insects produced for food and feed come with specific challenges. Insect researchers and breeders should thus aim to acquire in-depth knowledge on their physiology, metabolism, health, behaviour, and reproductive biology, and methods tailored for the species in question to enable genetic improvement through application of molecular genetics methods. Examples of key genetic information needed for effective insect management and breeding includes assessing population structure and evolutionary history, unveiling G × E interactions and which and how genes regulate the main production traits, identifying key immune system components and functions to prevent and combat infections and diseases, and resolving sex determination with the aim of manipulating population sex ratios. Research on genetics and fundamental biology are thus interdependent, and basic knowledge is still scarce for most production species, hence advancements in both domains are essential for progress in the field of insect breeding.

Genetic basis of production traits

Although many production traits are related to Darwinian fitness, not all evolutionarily relevant life-history traits are important in commercial production settings. This means that insights into the genetic basis of insect life-history traits obtained from fundamental research on insect models may only be partially transferable to production insects. Hence, the focus should be placed more on fundamental research on genetic regulation of commercially relevant traits. Study designs aiming to unravel the relationship between genotypes and phenotypes (the genotype-to-phenotype map) are recommended. Most traits of interest of farmed insects are related to their efficient production, such as simultaneous maximisation of growth and minimisation of food intake and development time. Similar to optimised feed conversion efficiency, many production traits are expected to be shaped by a complex interplay of factors that trigger numerous molecular pathways, and thus to be polygenic. Investigating such composite traits requires description of the trait components, including functional characterisation of involved organs and tissue, and applying quantitative genetic and genomic approaches to identify underlying trait architecture and regulatory networks of the involved genes. We encourage the community to take advantage of the many opportunities for generating large amounts of data, enabled by the technological advances, to make significant progress in elucidating the genetic basis of production traits. Further, consulting extensive research on several model insect species is advised to continuously complement our knowledge and inform future efforts.

Measuring phenotypes

Measuring phenotypes is a crucial aspect of farming insects and an important component of any selective breeding programme. It is not only a form of quality control or assessment of phenotypic progress but can also aid management by alerting on any potential problems in the production facilities. A hallmark of insect life cycles are the transitions between vastly different developmental stages, which require species-specific as well as stage-specific phenotyping methods. For example, flies and beetles are mostly harvested in their larval stage, whereas crickets and grasshoppers are reared through their juvenile nymphal stages until adulthood for harvest. Although measuring individual phenotypes of insects in large numbers and over time remains a challenge, we will benefit from using novel automated, sensor-based, and AI-assisted methods currently being developed to generate large and informative datasets in short time. In research or production settings, efforts should be made to obtain accurate trait measures to enable identification of genetic basis of production traits, provide insights into functional genetic correlations between traits, and ultimately inform breeding to ensures genetic progress. To improve breeding outcomes, we recommend leveraging species-wide population genetic data. Since genetic makeup influences trait expression and breeding response, focusing only on current production strains, often with unclear or comparatively narrow ancestry, may limit success. Consulting global population genetic inventories can enhance screening efficiency and reveal a broader range of valuable phenotypes.

Environmental effects, interaction with genetics and selection trade-offs

Given the major role environment plays in insect performance, not only control of its various relevant components in a production setting is crucial, as covered in several other dedicated BugBook articles, but also a profound understanding of how parameters need to be accounted for to mitigate impairment of efficient selection. Researchers and breeders are urged to keep environmental effects constant and reproducible when setting up selection experiments and measuring phenotypes. Another challenge in the field is scale, since general solutions for translating research findings from small numbers of insects in research laboratories to industrial contexts are virtually non-existent. To bridge the gap and extrapolate breeding progress from laboratory nucleus populations to production settings, we suggest installing replicate (quasi-)industrial facilities serving for experimental research in insect-breeding. Future approaches should also address the role and impact of environment- and scale-mediated epigenetic effects involved in plasticity responses of the various commercially farmed insects. Overlooking putatively important transgenerational non-additive genetic drivers could seriously hamper breeding progress.

Likewise, there is still a lot to be learned about the role of the environment in interaction with the genetics of insects for food and feed, which may in fact entail multiple levels of interactions, e.g. including microbiota possibly influenced by both diet and insect genetic background (Greenwood *et al.*, 2021; Silvaraju *et al.*, 2024). Optimising production tailored at market demands requires in-depth explorations of G × E interactions and their causal mechanisms. To ensure this, we champion the generation of in-depth knowledge on species-wide population structure, as well as on the evolutionary drivers underlying species-specific population genetic stratification. In some applied production contexts, selecting strains for maximising performance on a specific diet and otherwise constant conditions will increase economic revenue. Conversely, in settings that cannot be fully controlled or deliberately seek variable dietary substrates, generalist strains may outperform specialists, and/or allow less variable and thus more steady and predictable performance across a range of conditions. We stress that G × E interactions must be considered in breeding schemes tailored to industrial settings. Ignoring potential interactions can lead to important divergences from the expected results in the farm. In one scenario, the nucleus, centre of genetic improvement, must provide the multiplier and, ultimately, the production farm with genetically improved material. With the aim of increasing efficiency of the nucleus, the actual breeding environment is generally, although not advisably so, more favourable and/or controlled than the production site (control of abiotic conditions, diet quality, feeding regime, rearing density, pathogens and other stressors). For instance, with nucleus selection for improved conversion efficiency on concentrate feed, comparable trait gains are unlikely to be transferred to production settings using poor diets. Dedicated research is therefore needed to explore the transferability of genetic improvement to ensure selective breeding does not bypass actually decisive parameters, ultimately compromising long-term output of global farms.

Moreover, selective breeding is not an isolated process, where traits can be improved independently. Improvement in one specific trait leads to correlated

responses in others, either positive or negative, resulting in trade-offs. Both positive and negative correlations can be considered neutral, favourable, or unfavourable, depending on the context and may require adjusted management or product processing. For instance, reduced adult longevity with equal reproductive output upon increased larval biomass might be acceptable when larval protein is the production target, making this a negative, neutral correlation. Other trade-offs may unfavourably affect yield and need to be identified and carefully evaluated. A positive unfavourable correlation is exemplified when higher nitrogen conversion efficiency increases both larval growth and relative fat contents – population level fitness will likely increase, but aggravated lipid extraction may substantially affect product quality. Another potentially unfavourable correlation might result from selecting for increased antimicrobial peptide production in insects, which could be a boon for livestock feeding (Xia *et al.*, 2021). However, resource costly insect immune responses may compromise growth and population fitness (Joosten *et al.*, 2020), and affect public perception on welfare aspects (Kortsmit *et al.*, 2023).

Genetic monitoring and management of breeding populations

To improve and optimise insect breeding programmes and genetic management of populations, we emphasise genetic research shall also focus on unveiling genetic structure and thus relationships between subpopulations. Many of the genetic marker techniques developed for other organisms can readily be applied to quantify and monitor genetic variation in insects produced for food and feed. So far, much work has concentrated on a few species and populations/stocks. Based on improved insights into species-wide population stratification, future studies should ideally include a much wider selection of differentiated source populations to capture the true genetic scope of each species. The choice of methodology, sampling, and design should be tailored towards the goal of the application, and techniques and protocols should be developed and validated for the species and the aim in question. This calls for developing protocols for non-destructive genotyping of breeding candidates without compromising performance or reproductive success. Targeted genetic management, comparable to conventional livestock, requires a deep understanding of the evolutionary patterns, and ecological and demographic factors shaping population genetic structure across hierarchical levels, including wild populations and managed stocks. We opine that exploiting the genetic potential of a species as a whole is pivotal for a better understanding of genome-wide signatures of insect domestication and phenotypic consequences in a production context.

Data sharing

We encourage increasing transparency and data sharing through publications to advance the field and create long-term benefits for all breeders and the entire insect production industry. Further, future studies on the various non-genetic research disciplines related to insects as food and feed are urged to report basic genotyping data by applying available low-tech/cost genetic markers to help generating useful metadata. Specifically, reckoning that insect genetics has the potential to influence product quality up to performance at the next trophic level (see Sandrock *et al.*, 2022), continuing to ignore possible interactions between insect genetics and production parameters as well as product processing and/or features could turn out as a veritable shortcoming for the sector. In addition, the field would benefit from more openness on the research of genetics of farmed insects, for example by breeding companies. Technological advances in genetic analyses provide much opportunity for improving breeding programmes. Sharing experiences on shaping the research question and collecting and analysing phenotyping and genotype data will increase the likelihood of success. We recommend collaborative initiatives and coordinated international consortium efforts that will allow more ambitious research programmes, including larger scales (insect numbers), and enable tackling some of the major challenges to advance the field.

7 Conclusion

Despite the challenges discussed in this article, much progress has been achieved in the genetics of insects for food and feed. We believe that there is great promise for the continued development and improvement of research in this field. However, we also need to learn from the past in order to efficiently make progress. The advancement of technology not only pushed the boundaries of what can be achieved on model and non-model species but also changed the typical research bottleneck from generating genetic/genomic data to their analysis and providing accurate phenotypes associated with the genetic variation. Main challenges for the future include validating findings in small scale studies when upscaled to mass production conditions, assessing and

controlling the effect of environmental conditions and its interaction with genetics (and identifying the important drivers), characterising and exploiting beneficial genetic variation for trait optimisation, and balancing genetic improvement with inbreeding and maintaining genetic variation and robustness of cultures. Another point of focus is the huge differences among species (in life history, population structure, and evolutionary history) that can affect the outcome of applying various techniques and approaches. Importantly, claiming that the insect sector has understood how to avoid mistakes that were made in conventional livestock breeding deserves a responsible debate on controversial aspects like genome editing and insect welfare. Similarly, highlighting sustainability improvement as the sector's basic motivation deserves dedicated biodiversity considerations, which could range from protecting wild genetic resources from excessive intra-specific introgression to monitoring threats to ecosystems through inter-specific pathogen spillover or invasion potentials. Another veritable challenge is that a lot of knowledge, experience, and know-how exists among the many people working with insect for food and feed, however, much of this expertise is not published and thus not available for the broader research and industry communities. As a final note, we would like to mention that while the genetic improvement of farmed insects is still in its infancy, barely entering the domestication phase, genetics research and systematic breeding is undoubtedly the most powerful tool to further accelerate the transition to circular agriculture supported by insects. By unlocking their genetic potential, it is possible to enhance productivity, sustainability, and adaptability in insect farming. This represents a key opportunity to address global challenges in food security and environmental conservation, and there is an enormous amount of untapped insect diversity that may be exploited for food and feed production in the future.

Supplementary materials

Data is available on https://doi.org/10.1163/23524588-bja10260 under Supplementary Materials.

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