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REVIEW

Recent Advances in Population Genomics of Plant-Parasitic Nematodes

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ABSTRACT

Plant-parasitic nematodes are a costly burden of crop production. Ubiquitous in nature, phytoparasitic nematodes are associated with nearly every important agricultural crop and represent a significant constraint on global food security. Population genetics is a key discipline in plant nematology to understand aspects of the life strategies of these parasites, in particular their modes of reproduction, geographic origins, evolutionary histories, and dispersion abilities. Advances in high-throughput sequencing technologies have enabled a recent but active effort in genomic analyses of plant-parasitic nematodes. Such genomic approaches applied to multiple populations are providing new insights into the molecular and evolutionary processes that underpin the establishment of these nematodes and into a better understanding of the genetic and mechanistic basis of their pathogenicity and adaptation to their host plants. In this review, we attempt to update information about genome resources and genotyping techniques useful for nematologists who are thinking about initiating population genomics or genome sequencing projects. This review is intended also to foster the development of population genomics in plant-parasitic nematodes through highlighting recent publications that illustrate the potential for this approach to identify novel molecular markers or genes of interest and improve our knowledge of the genome variability, pathogenicity, and evolutionary potential of plant-parasitic nematodes.

Keywords: adaptation, cyst nematodes, evolutionary genetics, genome, genomics, plant resistance, population genetics, root-knot nematodes

The emergence and evolution of plant parasitism occurred several times independently in nematodes, resulting in a diversity of interaction modes with plants (Holterman et al. 2017). Because these organisms are microscopically small animals, they can appear to be less of a threat than they really are. However, plant-parasitic nematodes (PPN) are devastating pests because of their feeding habits and the role they play in spreading viruses and disease. The worldwide PPN impact has increased recently, probably also because of climate-challenging issues that aggravated PPN losses, which reached up to US$358 billion based on production figures and prices from 2010 to 2013 (Abd-Elgawad and Askary 2015). More than 4,100 species of PPN have been described to date (Decraemer and Hunt 2006) but only a few of them represent an important constraint on the delivery of global food security and, consequently, have attracted most of the research interest.

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Population genetics is a key discipline in plant nematology and more largely in phytopathology. It allows researchers to study genetic variations among and within populations by observing the distribution and frequencies of variants (i.e., alleles) in relation to the demography and history of the populations. Population genetics was applied to PPN in order to understand some aspects of their life strategies and, in particular, to improve our knowledge of the modes of reproduction, geographic origins, evolutionary histories, and dispersion abilities (Gilabert and Wasmuth 2013). However, population genetics studies with PPN have been hampered by the difficulties associated with sampling and the development of polymorphic molecular markers.

Most plant-parasitic nematodes have genomes of between approximately 50 and 100 Mb and can show different levels of ploidy. Aided by the availability of more genomes, plant nematologists recently scaled up methods and concepts of population genetics from a few markers to whole genomes. Few comparative genomics studies were conducted till now in nematodes, most probably because such analyses require high-quality genome assemblies. Comparative genomics usually consists of comparing gene content or genome variations for different species. Consequently, findings from comparative genomics studies

are often limited to evolutionary events from the distant past. To address evolutionary events occurring over much shorter and recent time periods, population genomics or a combination of population genomics and comparative genomics can be used. Like population genetics, population genomics allows the study of genetic variation among populations but using many more markers distributed across the entire genome. The present review aims to show the possible applications of population genomics, the results already obtained, and to foster such approaches in future studies.

To identify the research articles relevant for this review, we defined and combined two sets of queries. The first set included all of the most important plant-parasitic nematodes, based on their scientific and economic importance (Jones et al. 2013): "*Meloidogyne*" OR "*Globodera*" OR "*Heterodera*" OR "*Pratylenchus*" OR "*Radopholus similis*" OR "*Ditylenchus dipsaci*" OR "*Bursaphelenchus xylophilus*" OR "*Rotylenchulus reniformis*" OR "*Xiphinema index*" OR "*Nacobbus aberrans*" OR "*Aphelenchoides besseyi*". The second set, targeting population genomics, included two batches of keywords: "genomic*" OR "genomewide" OR "genome-wide" OR "genome wide" OR "genomescan*" OR "genome scan*" OR "metage*" OR "SNP*" AND "population*" OR "pathotype*" OR "race*". The 189 articles generated by a search on 24 August 2020 in the Web of Science core collection combining both sets of queries during the period 2008 to 2020 were analyzed and, after discarding irrelevant articles (i.e., mainly articles about resistance from the plant side), 31 articles were kept, in which different nematode populations or species were studied using genome or reduced-genome sequencing data. Note that running the second set of queries only (i.e., whatever the biological model) led to a list of 86,795 articles. Without any supplementary analysis of this too-long list, this showed that the most important PPN represented only 0.2% (i.e., 189 of 86,795) of the articles possibly dealing with population genomics. This same bibliographic analysis combined with the keyword "Caenorhabditis" led to 279 articles (i.e., 90 articles more than for PPN). Those additional queries showed that the shift from population genetics to population genomics was slow in our scientific community. However, the temporal analysis using the 189 articles (or only the 31 relevant articles) highlights three successive stages: on average, 10 articles per year from 2008 to 2014 (1 relevant per year), 20 articles per year from 2015 to 2018 (3 relevant per year), and 30 articles in 2019 (8 relevant) (Fig. 1), suggesting that the publication dynamic is becoming more and more active in this research domain.

FROM POPULATION GENETICS TO POPULATION GENOMICS

Even if the shift from population genetics to population genomics allows us to explore novel research questions, it must be noted that population genetics approaches are still relevant and advances were still obtained thanks to the genotyping of few microsatellite markers. The population genetic structure at different spatial scales has been described in an extended range of cyst nematodes (i.e., genera *Globodera* and *Heterodera*), including the potato cyst nematode species *Globodera rostochiensis* (Blacket et al. 2019; Handayani et al. 2020) and *G. pallida* (Thevenoux et al. 2020), the cereal cyst nematode *Heterodera avenae* (Wang et al. 2018), the soybean cyst nematode *H. glycines* (Wang et al. 2015), the carrot cyst nematode *H. carotae* (Esquibet et al. 2020; Gautier et al. 2019), and the beet cyst nematode *H. schachtii* (Gracianne et al. 2016; Kim et al. 2019).

Moreover, one limitation of population genomics in plant-parasitic nematodes is that genotyping cannot be conducted at an individual scale and, instead, has to be performed on pools of individuals; therefore, microsatellite genotyping was also used to explore the causes of the heterozygote deficiency and to estimate the effective population size in cyst nematodes. Montarry et al. (2015) showed that heterozygote deficiency is mainly due to consanguinity (mating between siblings) for univoltine species and to both consanguinity and substructure for polyvoltine species (i.e., species achieving several generations on its host plant). Through the estimation of the effective population size, a strong intensity of genetic drift was highlighted in *H. schachtii* (Jan et al. 2016) and *G. pallida* (Montarry et al. 2019). Those features of cyst nematode populations (consanguineous mating and low effective population size) are important to consider because they will, for instance, modulate the adaptation of nematodes to plant resistance.

Next-generation sequencing (NGS) has revolutionized the field of plant nematology in terms of the resources available to researchers and approaches used to investigate infection biology and understand the evolution of plant parasitism or plant adaptation. Most efforts in genome sequencing have been focused on species belonging to the orders Tylenchida and Aphelenchida, not only because of their major economic importance but also because of the practical ease of working with some of these PPN. Between 2011 and 2016, NGS allowed the genome sequencing of the pinewood nematode *B. xylophilus* (Kikuchi et al. 2011), the potato cyst nematodes *G. pallida* (Cotton et al. 2014) and *G. rostochiensis* (Eves-van den Akker et al. 2016), the potato tuber nematode *D. destructor* (Zheng et al. 2016), and the banana root nematode *Pratylenchus coffeae* (Burke et al. 2015). Since 2017, the number of available PPN genomes has almost doubled (source WormBase ParaSite) and at least 10 additional nematode genomes were published, including the soybean cyst nematode *H. glycines* (Masonbrink et al. 2019a), the stem and bulb nematode *D. dipsaci* (Mimee et al. 2019), *Rotylenchulus reniformis* (Showmaker et al. 2019), *R. similis* (Mathew et al. 2019; Wram et al. 2019), *G. ellingtonae* (Phillips et al. 2017), and several novel *Meloidogyne* spp. (Blanc-Mathieu et al. 2017; Koutsovoulos et al. 2020b; Phan

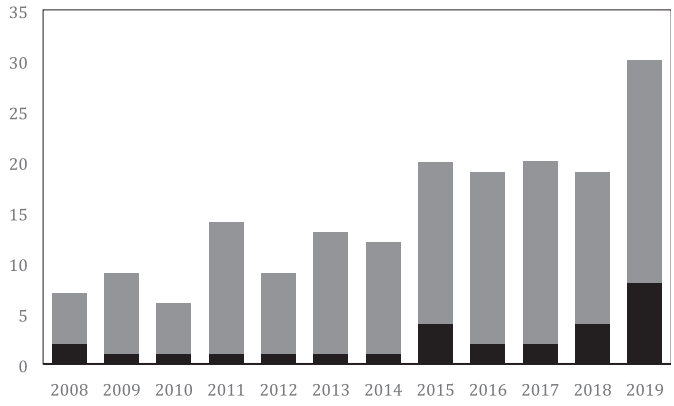

FIGURE 1

Year distribution of the publications obtained following the bibliographic analysis conducted for this review. The total number of research articles retrieved thanks to the bibliographic equation (gray bars) equals 189 articles across the period investigated. After discarding irrelevant articles, 31 articles (black bars) fully addressing a population genomics approach were kept and cited in this review.

et al. 2020; Somvanshi et al. 2018; Susič et al. 2020; Szitenberg et al. 2017). Furthermore, long-read technologies now allow the possibility of chromosome-scale resolution of PPN genomes, as was recently achieved for the soybean cyst nematode (Lian et al. 2019) and is planned in many ongoing projects.

Clearly, all of these NGS approaches open novel opportunities to understand population functioning and adaptation. In addition to improvement and confidence in the results obtained thanks to the use of thousands or hundreds of thousands of genetic markers, population genomics also allows researchers to address previously intractable research questions such as the identification of genes under selection, the identification of the mutations involved in adaptation to plant resistance, or the detection of meiotic recombination in parthenogenetic species. Population genomics represents an integrated strategy capable of simultaneously describing the genetic basis of phenotypic variation and describing the evolution of the underlying genes. Successful application of this strategy represents a novel approach for studying the genetics of adaptation, which is fundamentally about genetic variations within populations over time.

REVEALING GENETIC VARIATION AND RELATIONSHIPS AMONG POPULATIONS

New methods for PPN population genomics have also been developed during these five last years. Genome skimming was proposed by Denver et al. (2016). This pipeline includes low-coverage sequencing of populations or species of interest, de novo assembly, and search for orthologs of genes of interest from a predefined list from another reference genome. For example, this method can be used to detect the presence and to compare the sequence of genes coding for effectors (secreted proteins involved in parasitism) between different populations. This technique is relatively fast because it skips the gene prediction and annotation steps. However, it is still expensive if several populations need to be sequenced and require a preexisting list of targets, making it impossible to identify novel loci of interest.

Genotyping-by-sequencing (GBS) was first proposed to analyze PPN by Mimee et al. (2015). The GBS technique is based on sequencing a genomic subset following digestion by restriction enzymes. It allows the identification and characterization of single-nucleotide polymorphism (SNPs) over the entire genome, at very low cost, in numerous populations, and without a reference genome. These SNPs can then be used for genomewide association studies with virulence, bioclimatic variables, or any other population trait using classical bioinformatic tools. A similar technique called Nextera-tagmented reductively amplified DNA (nextRAD) was recently described by Khanal et al. (2019) to identify SNPs discriminating nematode populations from different geographic origins. GBS was successfully used to compare worldwide populations of *G. rostochiensis* and to retrieve the routes of introduction into North America, refining the previous portrait obtained using microsatellite markers (Boucher et al. 2013). Similarly, GBS was used to reveal the phylogenetic links between the new populations of *G. pallida* discovered in isolates from Idaho (United States) and the United Kingdom (Dandurand et al. 2019) or between the Peruvian, Chilean, and European *G. pallida* populations (E. Grenier and B. Mimee, unpublished data). Also, GBS analysis proved effective for investigating the genetic relationships among 64 North American *H. glycines* populations (Gendron St-Marseille et al. 2018). This study identified two distinct routes of differentiation within North America (northeastern and northwestern), both sharing genetic similarities to central (older) populations. Interestingly, a greater genetic differentiation was observed in more recent populations from Ontario whereas less diversity was expected owing to the founder effect. This is probably explained by the fact that central populations were submitted to a high selective pressure applied by the continuous use of resistant cultivars sharing a unique source of resistance (PI 88788). It is suggested that this may have led to large-scale homogenization toward virulent individuals within these central populations. Because this selective pressure is not yet widespread in the northeastern regions, the populations are still more genetically diverse and exhibit more variable phenotypes.

In a recent outbreak of the stem and bulb nematode *D. dipsaci* on garlic in eastern Canada, samples from 27 fields were compared using GBS (Poirier et al. 2019). Because these fields were suspected of having been contaminated by the same source over a short period of time, one did not expect to find much diversity between these *D. dipsaci* populations. In contrast, hundreds of variants were identified thanks to GBS and it was even possible to identify the probable source and the chronology of the infestations. These results highlight the potential of GBS for risk assessment (e.g., to improve our understanding of the routes of introduction and to rapidly identify, at low cost, populations that are potentially dangerous). A similar result was obtained by Mimee et al. (2015) when comparing numerous *G. rostochiensis* subpopulations from two fields only 50 km apart and from a common introduction. Only a few decades after the original introduction, GBS was able to identify several SNPs that discriminate the fields.

Although the root-knot nematodes (i.e., *Meloidogyne*) were the first plant parasites to have a whole genome sequenced, with *Meloidogyne incognita* in 2008 (Abad et al. 2008), population genomics analysis have only emerged recently. Using genome sequencing of a few *Meloidogyne* isolates from around the world, the level of variability between predicted protein-coding genes was estimated in *M. incognita*, *M. javanica*, *M. arenaria*, and *M. floridensis*, four parthenogenetic species with complex polyploid genome structures (Szitenberg et al. 2017). The analysis showed an overall low variability at the protein-coding level, suggesting a recent geographical expansion of these species. However, no attempt was made in this study to link these variations with biological traits, and variability in the noncoding part of genome was not considered. More recently, a population genomics analysis of 11 *M. incognita* Brazilian isolates showed low variability in terms of SNPs and short indels at the whole-genome scale between these populations (approximately 0.2% of the positions) despite their differences in terms of host compatibility and geographical origins (Koutsovoulos et al. 2020a). Despite the low variability, fixation index (F_{ST}) values computed between these populations were all high, showing a lack of genetic exchange, as expected for purely parthenogenetic species. The addition of eight other populations, including five from the United States, two from Africa, and one from the Caribbean, did not yield more variable positions in the genome, further supporting a recent expansion most likely due to human activities such as agriculture (Koutsovoulos et al. 2020a). Performing a principal component analysis using SNPs identified between the isolates, whole genomes showed a distinct separation in three clusters: one containing Brazilian and North American samples, one containing Brazilian and other diverse geographical samples, and one containing only Brazilian samples. The addition of 48 Japanese *M. incognita* isolates substantially expanded the geographical sampling but this did not alter the grouping because all of these new isolates were placed within the previously described cluster containing the North American and some Brazilian isolates (Asamizu et al. 2020). Similar results were also reported for another *Meloidogyne* sp., *M. graminicola*, after analysis of 12 mitogenomes corresponding to worldwide populations of this species. Once again, the low genomic diversity observed within the studied populations strongly suggests a recent expansion of *M. graminicola* in Southeast Asia (Besnard et al. 2019).

To assess whether variable genomic regions could differentiate some *Meloidogyne* spp. and populations present in South Africa (*M. incognita*, *M. javanica*, and *M. enterolobii*), an analysis coupling pool-seq and GBS was performed (Rashidifard et al. 2018). It allowed the identification of common SNPs across these

species, and analysis of the frequencies and distribution of these SNPs enabled the separation of the different species and identification of species-specific variants.

Target enrichment sequencing has recently appeared as a novel approach for future population genomics studies. Target enrichment focuses on selectively capturing, enriching, and sequencing only the targeted genomic regions. A pathogen-enrichment sequencing (PenSeq) approach was developed and used to explore population and race diversity in oomycete pathogens (Jouet et al. 2019; Thilliez et al. 2019). PenSeq provides a cost-effective and high-throughput approach to study sequence polymorphisms. In PPN, this approach was very recently used to address the basis of adaptation to plant resistance on a population scale in the potato cyst nematode *G. pallida* (Varypatakis 2019). PenSeq was adapted and applied to capture and sequence candidate effectors from selected *G. pallida* experimental lineages obtained on different resistant potato cultivars. Analyses showed the presence in the four investigated lineages of 309 variants which, in total, represent 122 different annotated genes containing at least one SNP.

UNDERSTANDING PLANT-PARASITIC NEMATODE BIOLOGY AND REPRODUCTION

Plant-parasitic nematodes display a range of different reproduction modes. Curiously, some of the most devastating species such as *M. incognita* and other tropical root-knot nematodes have been described as reproducing without sex and meiosis (i.e., mitotic parthenogenesis). Asexual reproduction is usually considered to limit adaptability because of the absence of recombination and, thus, to constitute an evolutionary dead end in animals (Castagnone-Sereno and Danchin 2014). However, strictly asexual reproduction was inferred by cytogenetics observations performed by Triantaphyllou (1981, 1985) more than 30 years ago and never further confirmed by other investigations. By comparing the whole genomes for 11 *M. incognita* isolates, a density of SNP markers sufficient to test for evidence of recombination in this species was obtained (Koutsovoulos et al. 2020a). SNPs were used as markers to perform genetic tests such as linkage disequilibrium and the four-gamete test. The profile of linkage disequilibrium and the proportion of pairs of markers passing the four-gamete test as a function of intermarker distance showed no evidence for sexual recombination in this species. Analysis of the variability at synonymous and nonsynonymous sites in coding regions across the isolates also showed a reduced efficiency of selection, consistent with the lack of outcrossing in this species. The same genetic tests, however, showed clear signatures of meiotic recombination in the cyst nematode *G. rostochiensis*, used as a control. Independent confirmation of the lack of genetic exchange between populations in *M. incognita* is striking given its adaptive potential.

The mechanisms underlying this adaptability in the absence of sexual reproduction remain elusive, thus far. Because only low levels of genomic variations at the SNP and short-indel level were observed between *M. incognita* isolates, other types of mutations were investigated. The pool-seq data generated by Koutsovoulos et al. (2020a) was used to investigate mutations spanning longer suites of nucleotides across the *M. incognita* Brazilian populations as well as the Mexican population (from Morelos) that was initially used to produce the reference genome (Blanc-Mathieu et al. 2017). In this analysis, variations in presence frequencies of transposable elements (TEs) across loci at the whole-genome level were investigated (Kozlowski et al. 2020). A phylogenetic analysis of the *M. incognita* isolates based on their pattern of TE frequency variations yielded the exact same topology as the one previously obtained with short-scale variations (Koutsovoulos et al. 2020a), indicating that most of the changes in TE frequencies followed the nucleotide-level divergence between isolates (which itself showed no correlation with the biological traits investigated). Interestingly, some variations in frequencies did correspond to TE neo-insertions, some of which were in coding or possibly regulatory regions. Overall, this analysis showed that TE activity contributes substantially to the genomic plasticity and diversification in *M. incognita*. Furthermore, surprisingly, variability in TE presence frequencies was also observed within populations, indicating heterogeneity between individuals, despite each of the isolates being reared from the offspring of one single female.

The population genomics analyses conducted till now on apomictic *Meloidogyne* spp., albeit showing overall low variability at the SNP level, allowed confirmation of the absence of genetic exchange between populations in *M. incognita* and revealed surprising sequence variations within isolates.

UNDERSTANDING PLANT-PARASITIC NEMATODE PATHOGENESIS AND ECOLOGY

The terms "race" and "pathotype" were routinely used for years to express differences in host specificity in nematology. A distinction was proposed by Sturhan (1985), in which pathotypes were rather defined as "virulence phenotypes" and "races" as a population or group of populations which differs from others by the presence, absence, or frequency of their genes or alleles. Hence, whereas "race" is a population concept, "pathotype" should rather correspond to genotypes shared in different populations, meaning that some populations may exist as one pure pathotype only, while others will consist of several pathotypes or phenotypes at different frequencies. Important results were recently achieved thanks to population genomics that have either supported or not supported the concepts of root-knot nematode races and cyst nematode pathotypes.

In the recent study published by Koutsovoulos et al. (2020a), 11 Brazilian populations of *M. incognita*, presenting four distinct patterns of host compatibility (commonly referred to as host races), were compared. The genomewide single-nucleotide variations between these isolates showed no significant correlation with the biological traits considered; namely, the geographic distribution, patterns of host compatibilities, and the currently infected host plant. The lack of correlation with the geographic distribution was also supported by isolation by distance analyses. Overall, this population genomics analysis revealed that no phylogenetic signal was underlying the so-called host races and that the term "race" is biologically incorrect because it does not correspond to monophyletic groups. These results also suggested that the different patterns of host compatibilities most probably resulted from multiple independent gain and loss of abilities to parasitize certain hosts. Looking more closely at the pattern of compatibility of 48 *M. incognita* Japanese isolates on five different sweet potato cultivars, Asamizu et al. (2020), defined six patterns of host compatibilities and set out to uncover genomic variations associated with these patterns. Using population genomics approaches, a genomic region spanning 1 Mb was identified as associated with the six patterns of compatibilities with sweet potato cultivars, though the rest of the genomic regions showed very little variation. Hence, although no clear relationship could be identified between single-nucleotide variations at the whole-genome level and the patterns of host compatibility on four different host plants in *M. incognita*, analysis of different cultivars of the same plant species (sweet potato) allowed identification of a genetic background underlying the differences of infection patterns in the Japanese isolates.

In the potato cyst nematode species *G. pallida* and *G. rostochiensis*, resistance-breaking populations were revealed and attempts were made to develop formalized schemes for potato cyst pathotypes nomenclature and characterization. According to the Kort et al. (1977) classification, three pathotypes of *G. pallida* (Pa1,

Pa2, and Pa3) and five of *G. rostochiensis* (Ro1, Ro2, Ro3, Ro4, and Ro5) have been recognized in Europe. However, subsequent investigations showed that there is no clear distinction between some Pa2 and Pa3 populations in relation to their virulence against the two main resistance sources (*Grp1* derived from *Solanum vernei* and *H3* derived from *S. tuberosum* subsp. *andigena*) and that the variation in multiplication levels is rather explained by environmental conditions (Blok et al. 1997; Phillips and Trudgill 1998). These pathotypes are often jointly referred as Pa2/3. Although there have been several attempts to distinguish the virulence of populations by means other than bioassays, there is currently no reliable molecular method of classification, which presents a barrier to effective management of potato cyst nematodes.

In 2015, Eves-van den Akker et al. (2015) applied NGS to the sequencing of mitochondrial markers and conducted a metagenetic approach to identify *G. pallida* diversity at the country level. The analysis of 687 Scottish field populations revealed the presence of three mitotypes, one of them being found only in Pa1 pathotypes. More recently, a population genomics study using GBS confirmed that samples belonging to the Pa1 pathotype can be distinguished from the rest of the European *G. pallida* populations (Dandurand et al. 2019). These Pa1 populations showed a common genetic signature despite different geographical origins, supporting the development of a diagnostic tool. Conversely, no significant distinction could be established between the Pa2 and Pa3 populations but, rather, the study highlighted regional differences in their genomic sequences.

GBS was also used to identify SNPs, allowing researchers to distinguish potato cyst nematode variants or groups that strongly differ in their development rates on a set of resistant potato cultivars. To this end, 18 worldwide *G. pallida* populations, including representatives of the three pathotypes but also North Peruvian and Chilean populations, were sequenced by GBS and phenotyped on three resistant potato genotypes (two harboring the *S. vernei* and one the *S. andigenum* resistance). Among the 3,132 SNPs analyzed, 70 were found to correlate with the levels of development on *S. vernei* resistant potato and 45% of them corresponded to nonsynonymous mutations in exons (E. Grenier and B. Mimee, unpublished data). When looking at the population groupings obtained using these SNPs of interest, no clear match with the pathotypes was observed but the main distinction was coherent with the phylogeographic history of *G. pallida*.

In 2015, Palomares-Rius et al. (2015) reported the first study of genomewide variation in populations of the pinewood nematode *B. xylophilus*. Six populations isolated from different parts of Japan and showing different phenotypic traits were sequenced, and results showed that the level of diversity in the genome was high (4.1% of the genome positions were variable as SNP or small indels), suggesting multiple introduction of this invasive species into the country. This was confirmed using the sequencing of mitochondrial genomes of 285 individuals from 12 Japanese populations, which clearly identified two genetic clades (Zhang et al. 2018). Moreover, Palomares-Rius et al. (2015) identified a gene set particularly affected by genomic variations, and the functional annotation of those genes indicated that they had potential roles in pathogenesis. Soon after, transcriptome and genome sequences of strongly and weakly aggressive populations (i.e., differing by their reproductive and growth ability) were analyzed (Ding et al. 2016). Results demonstrated that *B. xylophilus* populations showed dissimilar expression patterns on both the transcript and exon levels, and several SNPs were identified as potential genetic markers able to distinguish the strongly and weakly aggressive populations. Four of them were experimentally validated on other populations, providing potential diagnostic tools to facilitate the control of pine wilt disease (Ding et al. 2016).

Apart from the advances obtained regarding nematode pathogenesis, population genomics was also used to detect genetic loci under selection associated with environmental and climatic parameters (Gendron St-Marseille et al. 2018). Three Bayesian methods were used to identify outlier loci: BayPass (Gautier 2015), BayeScan (Foll and Gaggiotti 2008), and BayeScEnv (de Villemereuil and Gaggiotti 2015). Across 64 North American populations, 15 outlier loci were identified and most were found to correlate with temperature. The genic environment around these loci was further explored by whole-genome sequencing of four representative populations, allowing the identification of high-impact variants in coding regions that appeared to be under strong selection. These genes were mostly related to chemosensory functions such as foraging and response to stress. However, these adaptations appeared to be localized to specific regions and, overall, this study showed that no critical adaptive events were required by *H. glycines* to establish in northern latitudes.

UNDERSTANDING ADAPTATION TO THE HOST AND IDENTIFYING GENES UNDER SELECTION

Identification of the genes involved in the adaptation of plant-parasitic nematodes to plant resistance represents a major challenge owing to the complexity of their genomes and life cycles and a lack of tractable model organisms. The level of diversity within effector families and the identification of genes under diversifying or positive selection have been used to predict their involvement in plant parasitism. The rationale for focusing on such genes is that products operating at plant–pathogen interfaces will display an accelerated evolution. Since 2009, two genes from the SPRYSEC gene family and one gene from the VAP gene family were shown to be involved in incompatible plant–nematode interaction and to interact with plant proteins (Lozano-Torres et al. 2012; Rehman et al. 2009; Sacco et al. 2009).

Using a genome scan approach, two studies were recently conducted regarding the identification of genomic modifications associated with *G. pallida* adaptation to the resistant potato cultivar Iledher obtained from the *S. vernei* resistance source (*Grp1*). The first study was a low-density genome scan using 53 microsatellite markers, which only validated the feasibility of such an approach on biological material coming from experimental evolution (Eoche-Bosy et al. 2017a), and the second was a high-density genome scan using SNP markers (Eoche-Bosy et al. 2017b). Altogether, these studies highlighted the great advantage of a population genomics approach using whole-genome sequencing compared with a population genetics approach using fewer markers. The genome scan relies on the fact that selection affects only parts of the genome, in contrast to other evolutionary forces (mutation, migration, and genetic drift) that affect the genome in its entirety. When a mutation for virulence is selected, this selection will leave a genomic signature that also affects the neighboring loci. Identifying these regions under selection is then possible by calculating and comparing the genetic differentiation at each marker between unselected and selected populations. The final step is to compare the genetic differentiation calculated for each marker with a neutrality envelope, which can be simulated by different models. Markers outside the neutrality envelope are called outliers and, therefore, are indicative of genomic regions putatively affected by selection. In these genomic regions, a decrease of the genetic diversity is also expected and, therefore, it is a second statistic that can be used when searching for footprints of selection in a genome (Luikart et al. 2003; Storz 2005).

Eoche-Bosy et al. (2017b) performed a whole-genome resequencing of pools of 300 individuals coming from two independent pairs of virulent or avirulent lineages evolved on the resistant cultivar Iledher and on the susceptible Désirée, respectively. The reads were mapped against the reference genome of *G. pallida* (gpal.v1.0) (Cotton et al. 2014) to identify SNP markers, and the allelic frequencies at 1.6 million SNPs were used to perform the

genome scan. A first list of 275 SNPs that were detected as outliers was obtained using the package BayPass (Gautier 2015). Among the 275 outliers, 31 showed a decrease of the genetic diversity in both virulent lineages (based on the LnRH test) (Kauer et al. 2003; Schlötterer and Dieringer 2005). Among those 31 good candidates, 12 SNPs were located in genes, including 5 in exons. Overall, the candidate regions were enriched in genes coding for secreted proteins and SPRYSEC in particular. Recently, thanks to a new genome assembly produced by Wageningen University in 2019 (HR-MATCH project funded by Netherlands Organization for Scientific Research) that—based on primary genome statistics—can be considered a better reference genome, this genome scan analysis was rerun and ended with a total of 302 outlier SNPs, including 32 showing a decrease in genetic diversity. However, it appeared that only 8 of the 31 and 32 genes were common to both analyses (Grenier et al. 2020).

Castagnone-Sereno et al. (2019) carried out a genomewide comparative analysis of virulent versus avirulent lineages of *M. incognita* relative to the *Mi-1* resistance gene in tomato. The virulent lineages were previously independently derived from two different avirulent populations by experimental evolution. The two virulent lineages were compared with their avirulent lineages of origin to evaluate gene copy number variations (CNV) associated with the switch from avirulence to virulence. Although this analysis did not involve genome sequencing but, instead, microarray-based comparative genomic hybridization, this allowed monitoring of CNV at the whole-genome scale. Convergent CNV (mostly gene copy losses) between the independently emerged virulent lineages as compared with their avirulent populations of origins were identified. Hence, some genome regions underwent the same variations in copy numbers (mostly losses) independently in different populations as a response to selection by a resistance-gene-bearing tomato cultivar. Moreover, this study supports the view that gene loss could be an important class of adaptive genetic mechanism in this parthenogenetic species and, more largely, in clonal animals.

To date, there has been relatively low success in identifying genes under selective pressure in *H. glycines* owing, in part, to the complexity of virulence mechanisms and to the repetitive nature of the genome (Mitchum 2016). Lacking genomic infrastructure, the first study investigated the possibilities of a genomewide SNP discovery approach to detect virulence genes between two population biotypes (Bekal et al. 2008). More recently, a genetic mapping study using an allelic imbalance approach between two *H. glycines* populations was used to identify SNPs linked to virulence (Bekal et al. 2015). Two putative virulence genes tightly linked to virulence were identified: a bacterial SNARE-like protein (HgSPL-1) and a Biotin synthase (HgBioB). Yet, generalizing these findings to other populations has proven challenging, with copy numbers of HgSPL-1 varying between isolates of the same populations and between virulent populations. Additionally, an orthologous gene annotation analysis comparing transcriptomic data to nine related PPN expended the *H. glycines* effectorome by highlighting 3,324 putative effector genes sharing structural similarities to known effectors of PPN (Masonbrink et al. 2019b). Currently, single-individual-based transcriptome amplification is being used to identify genes under selection for virulence in PPN (Ste-Croix et al. 2021). This study investigated differential gene expression across eight populations of *H. glycines*, with individuals selected based on their virulence phenotypes against resistant soybean Peking and PI 88788 and contrasted to an avirulent reference group. In these contrasts, Ste-Croix et al. (2021) identified what seems to be a multifaceted strategy against Peking resistance, involving many genes and multiple biological mechanisms. In comparison, virulence to PI 88788 appeared to mostly involve selective gene regulation.

Further analysis revealed a diverse set of mechanisms at play in *H. glycines* virulence, including gene hitchhiking with TE, gene diversification through duplication, and horizontal gene transfer events. The later of theses mechanisms was found to be prevalent in *H. glycines,* with 107 horizontal gene transfers not reported in other PPN, and likely contributed to the evolution of virulence (Masonbrink et al. 2019a). These genomic insights culminated in the release of the online open-access soybean cyst nematode genome browser, which integrates genomic and transcriptomic data from different studies as well as structural and functional analysis of genes (https://scnbase.org/) (Masonbrink et al. 2019b).

The first interspecies comparisons using multiple populations were also recently carried out in order to better understand the genomic basis of host adaptation in the *Globodera* genus. Sabeh et al. (2019) compared the transcriptome profiles of populations from closely related species *G. rostochiensis* versus *G. tabacum* and *G. pallida* versus *G. mexicana*, in which only the first species of each pair is able to develop on potato. Although all of these species shared a very similar genome sequence, exposure to potato root exudate resulted in a distinct transcriptomic profile in potato cyst nematode species, highly enriched in effector proteins sequences. This study also revealed seven transcripts that were unique to all potato cyst nematode species. Such comparisons at the population level, using several species, open the way to a better understanding of phenotypic variation in complex traits such as host specificity or host adaptation.

PERSPECTIVES

Sequencing the whole genome of a single PPN individual was, until recently, impossible for most of the species. PPN, being obligate biotrophs, are difficult to rear, and the nucleic acid content of a single individual is not sufficient for sequencing (Serra et al. 2018). To deal with this limitation, the population genomicists working on PPN have to use pooled individuals for sequencing, which causes the loss of haplotype information and prevents the use of several genetic indicators and the evaluation of linkage disequilibrium. Recently, a "single-cell" sequencing approach to study gene expression in individual nematodes was proposed (Chang et al. 2021; Serra et al. 2018). This method, based on the Smart-seq2 protocol (Picelli et al. 2013), uses a preamplification step of the cDNA libraries prepared from very low RNA inputs. This method provides perspectives to better estimate population allele frequency and detect rare variants, and opens the way for other genotyping analyses (e.g., multilocus genotyping) that are not possible using pooled samples.

Acquisition of genes important for parasitism from bacteria, fungi, and other nonmetazoan via horizontal gene transfer seems to be a hallmark of plant-parasitic nematodes (Danchin 2016; Haegeman et al. 2011). The genes seem to have been functionally assimilated in nematode genomes, with many of them being expressed during parasitism and the protein product being found in nematode secretions. Furthermore, the GC content and codon usage of the acquired genes are not different from the rest of the genes in the nematode genomes. Because several of these genes exist now as a multigene family, it has been hypothesized that selection has favored individuals bearing multiple copies of these genes (Danchin et al. 2010). However, whether these genes are actually under diversifying selection across nematode species and populations has never been tested, and population genomics will allow such important analysis to be undertaken.

Progress in the throughput of genome sequencing technologies associated with a continuous reduction in costs have recently fueled population genomics in PPN. Long-read technologies aided by scaffolding methods such as optical mapping or chromatin contact information (Hi-C, 3C, and so on) allows researchers to expect

high-quality reference genomes for a diversity of plant-parasitic nematode species in the near future. This will positively affect the resolution of population and comparative genomics analyses, enabling the study of linkage information at long range. Recent bioinformatics and technical progress also allows separate phasing and assembly of the different haplotypes of diploid and polyploid genomes (Aleman 2017; Weisenfeld et al. 2017), providing a more realistic representation of a genome than ever before. There is no doubt that population genomics will benefit this progress, too.

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