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Tracking invasion and invasiveness in Queensland fruit flies: From classical genetics to 'omics'

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Abstract Three Australian tephritid fruit flies (*Bactrocera tryoni* – Q-fly, *Bactrocera neohumeralis* – NEO, and *Bactrocera jarvisi* – JAR) are promising models for genetic studies of pest status and invasiveness. The long history of ecological and physiological studies of the three species has been augmented by the development of a range of genetic and genomic tools, including the capacity for forced multigeneration crosses between the three species followed by selection experiments, a draft genome for Q-fly, and tissue- and stage-specific transcriptomes. The Q-fly and NEO species pair is of particular interest. The distribution of NEO is contained entirely within the wider distribution of Q-fly and the two species are ecologically extremely similar, with no known differences in pheromones, temperature tolerance, or host-fruit utilisation. However there are three clear differences between them: humeral callus colour, complete pre-mating isolation based on mating time-of-day, and invasiveness. NEO is much less invasive, whereas in historical times Q-fly has invaded southeastern Australia and areas of Western Australia and the Northern Territory. In southeastern fruit-growing regions, microsatellites suggest that some of these outbreaks might derive from genetically differentiated populations overwintering in or near the invaded area. Q-fly and NEO show very limited genome differentiation, so comparative genomic analyses and QTL mapping should be able to identify the regions of the genome controlling mating time and invasiveness, to assess the genetic bases for the invasive strains of Q-fly, and to facilitate a variety of improvements to current sterile insect control strategies for that species [*Current Zoology* 61 (3): 477–487, 2015].

Keywords *Bactrocera*, Genomics, STERILE-insect-technique, Interspecific-hybridisation

1 Introduction

Successful biological invasion requires a novel environment that has appropriate cues, resources, and physical conditions, coupled with competition at sufficiently low levels to allow dispersal, survival, and reproduction. Attempts by humans to halt invasions are based on lowering one or more of dispersal, survival and reproduction in the invaded area, by either reversing one or more of the cues, resources or conditions (Lodge, 1993), or by interfering directly with the invader. Each aspect of invasion and control is influenced by genes, so genetics can help us understand the natural process of invasion, and how to control it. An ideal study system would be one where closely-related strains or species differ very little, except in their invasion ability, so that the critical genetic differences that relate to invasiveness might be pinpointed relatively easily.

Three endemic tephritid fruit flies in Australia (the Queensland fruit fly *Bactrocera tryoni* "Q-fly", the lesser Queensland fruit fly *Bactrocera neohumeralis* "NEO" and Jarvis' fruit fly *Bactrocera jarvisi* "JAR") are potentially informative models for genetic study of pest status and invasiveness. Q-fly and JAR manifest a range of morphological and behavioural differences, including clear differentiation in host-fruit preference, temperature tolerance and pest status. However Q-fly and NEO display extraordinary ecological and genetic similarity, although they differ in their mating time of day and their invasiveness. This article shows how genetics and genomics are being applied to these species to elucidate the molecular bases of their respective pest and invader phenotypes, and to improve current sterile insect technology for the control of Q-fly.

2 Ecology: Distribution, Differentiation, and Invasiveness

Historically, the distribution of NEO during the winter months was found to be restricted to a narrow coas-

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tal belt extending as far south as Coffs Harbour, New South Wales (NSW) (May, 1963). The range of NEO is contained entirely within the wider and expanding distribution of Q-fly (Fig. 1) (Osborne et al., 1997) although, during the summer months, there is temporary expansion of the NEO range, such that this species has been trapped occasionally as far south as Sydney (Gillespie, 2003) and as far west as Emerald, Clermont, Gayndah and Mundubberah Queensland (May, 1963; Hancock et al., 2000; Lloyd et al., 2010; Royer and Hancock, 2012). Compared to Q-fly, the population density of NEO usually appears to be lower, based on a large scale trapping experiment in Feb 1994 (Osborne et al., 1997). Within its range, NEO seems to be most abundant in tropical-subtropical regions that have a high relative humidity and mild winters.

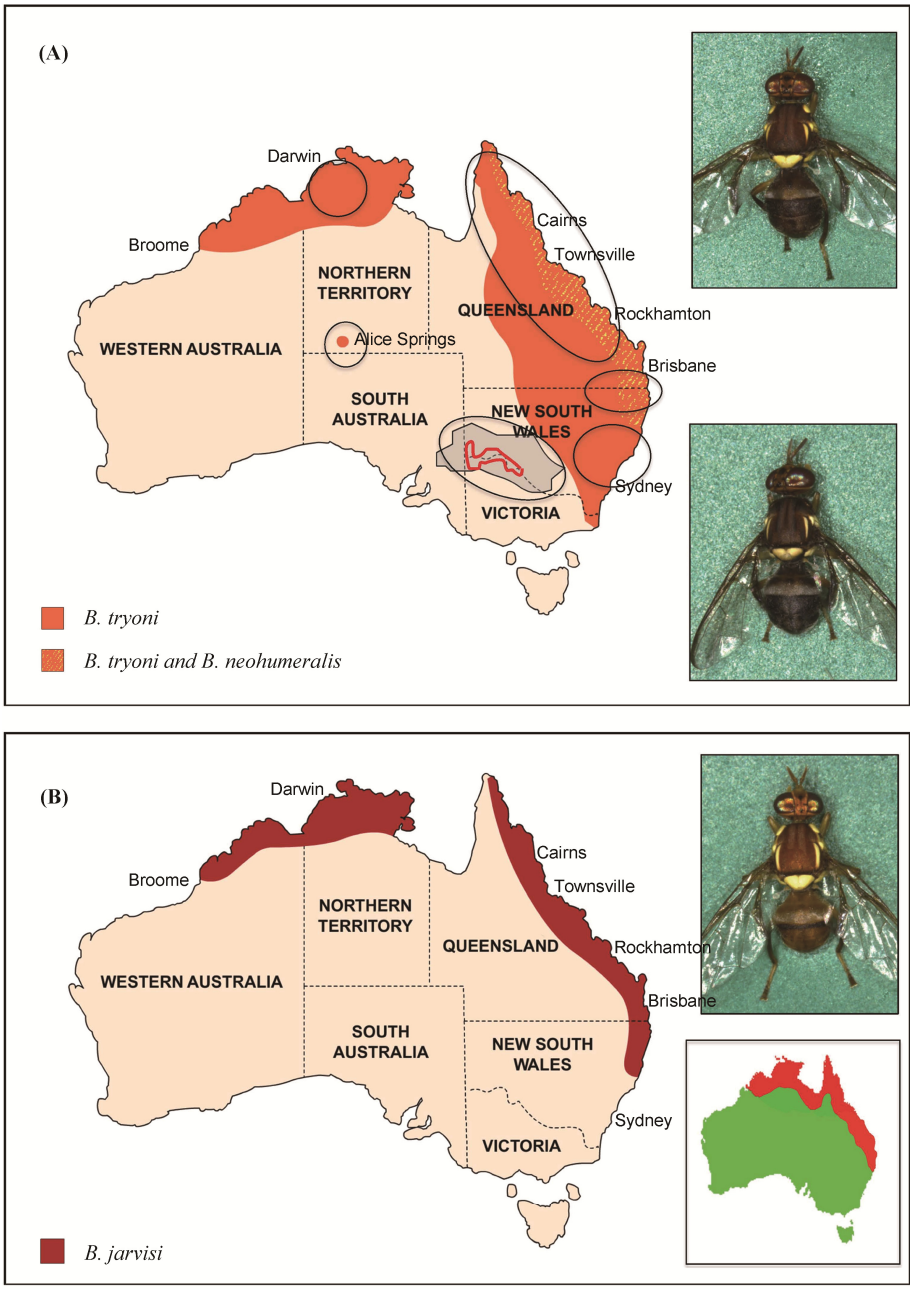

Fig. 1 The distributions of *B. tryoni*, *B. neohumeralis* and *B. jarvisi* in Australia. From Raphael et al. (2014).

A. The distribution of *B. neohumeralis* is entirely within the broader distribution of *B. tryoni*. The ovals show the populations of *B. tryoni* that are differentiated by microsatellite analyses. Grey shading is the FFEZ (the former Fruit Fly Exclusion Zone). The red outline shows the Greater Sunraysia Pest Free Area. Top right, *B. tryoni*, bottom right, *B. neohumeralis*. **B**. The distribution of *B. jarvisi* and, inset, the distribution of its native host the Cocky apple (*Planchonia careya*). Top right, *B. jarvisi*

The two sibling species Q-fly and NEO are easily distinguished by the colour of the humeral calli ('shoulder pads') (Drew, 1989). Despite considerable study, a difference in mating time remains the only known isolating mechanism between these two species. Q-fly mates in a narrow window of falling light intensity at dusk (like most *Bactrocera* species), whereas NEO mates in bright light during the middle of the day (Tyschen and Fletcher, 1971; Smith, 1979; Pike et al., 2003). This difference persists over decades in the laboratory, and explains the lack of hybrids in the species' shared natural range, where wild hybrids are not detectable by microsatellite analyses (Gilchrist and Ling, 2006).

There is a long history of ecological and physiological studies of Q-fly and NEO, because Q-fly is Australia's most serious horticultural pest, and a major target for domestic and international quarantine efforts (Drew, 1989; Clarke et al., 2011) In these studies, the two species have shown no differences in pheromones (Bellas and Fletcher, 1979), temperature tolerance (Meats, 2006), or utilisation of a wide range of host fruits – larvae of the two species can even emerge from the same piece of fruit (May, 1953; Gibbs, 1967; Drew, 1989; Hancock et al., 2000). Apart from their mating time difference, the only clearly established ecological difference between the two species is that Q-fly is more invasive than NEO (Drew, 1989; Sved et al., 2003) and has followed the spread of horticulture through eastern Australia (Meats, 1981), including into drier and cooler areas beyond its native habitat (Fig. 1). In historical times Q-fly has regularly appeared in Victoria and South Australia, and there has also been sporadic invasion of Western Australia and Northern Territory (Dominiak and Daniels, 2012). Because of the close ecological similarity of Q-fly and NEO, it is unclear what phenotypic differences might underly the difference in invasiveness. A summary of what is known about Q-fly's habitat requirements and dispersal ability shows that there are many uncertainties yet to be cleared up for that species (Clarke et al., 2011), let alone deciding what are the critical differences between Q-fly and NEO.

In contrast, the invasiveness of JAR is better understood – its distribution (Fig. 1B) closely follows that of its preferred host fruit: cocky-apple *Planchonia careya*, suggesting that it is limited by its host-preference. However, in areas where horticulture has replaced the native hosts, JAR has been found to infest fruit crops such as mangoes, and has moderate pest status (Smith et al., 1988). JAR has been recorded from 83 wild and commercial hosts from 28 families (Hancock et al., 2000), and the increasing number of hosts from which it is isolated suggests that its host range might now be increasing (Smith et al., 1988). In fact all three species might continue to change their range as climate change proceeds (Clarke et al., 2011).

3 Opposing Invasion: Past Control Attempts

Control of Q-fly requires strategies that are acceptable to both conventional and organic agriculture. Traditional pest control uses insecticides, but increasing insecticide bans (Dominiak and Ekman, 2013) create the need for more environmentally acceptable controls (Spinner et al., 2011; Langford et al., 2014). Many methods show some promise but require further research (Clarke et al., 2011). However the only current non-insecticide option that has been applied on a large scale against Q-fly is the Sterile Insect Technique (SIT), which has already been used to eradicate *Bactrocera cucurbitae* from the Okinawa islands (Kakinohana et al., 1997) and is currently used for management of the Mediterranean fruit fly, *Ceratitis capitata* (Virdi, 2014). SIT involves the area-wide release of millions of sterile males, whose mating with wild females results in reduced abundance of the wild population (Bourtzis and Robinson, 2006). In contrast to insecticides, SIT is species-specific. Also, development of resistance to SIT would require alteration to some part of the mating system, and it has been suggested that this might possibly be a slower process than evolving an alteration to the target site of a detoxifying enzyme for a new chemical pesticide. Disadvantages of SIT include the expense and technical difficulties of establishing and maintaining a mass-rearing facility, the need to mark released flies to distinguish them from wild flies in the field, the effects of radiation used to sterilise the males, and the reduced fitness of mass-reared flies in the field due to laboratory adaptation. The latter has been documented in Q-fly (Meats et al., 2004; Dominiak et al., 2008; Gilchrist et al., 2012; and in other insects (Bush et al., 1976; de Souza et al., 1988; Economopoulos, 1992).

SIT has been employed annually against Q-fly in temperate fruit growing regions of New South Wales, Victoria and South Australia, formerly designated the Fruit Fly Exclusion Zone (FFEZ). However, those SIT campaigns have generally failed to produce measurable results. Since 1964, eleven separate SIT trials have been mounted against Q-fly. With one exception (in 1995 in Perth), all failed to show any effect on pest populations

(Andrewartha et al., 1967; Bateman, 1991; Fisher, 1996; Meats, 1996; Meats et al., 2003a; Paton, 2003). Outbreaks of Q-fly now occur most years within the FFEZ.

There are a number of reasons for the failure of those SIT efforts. First, the success of SIT depends on wild females being mated by the sterile males, which must therefore survive to and beyond breeding age. However, this survival depends on the prevailing climatic conditions (Meats et al., 2003a), which are very adverse in the locations of the programs (inland NSW, inland Victoria and Adelaide) where there are hot, dry summers. This suggests that it might be best to concentrate SIT efforts on any refuge localities that might be in or near the control area, but with milder summer conditions. Second, the Australian SIT efforts have been carried out with very small numbers of sterile insects. The weekly production of only 12–15 million sterile Q-fly at the Fruit Fly Production Facility (FFPF) located at Camden, NSW (Dominiak et al., 2008) is supposed to support SIT in three states. By contrast in Japan, weekly production of sterile *B. cucurbitae* for the much smaller Okinawan islands alone is over 100 million flies per week, and this is merely for prophylactic SIT programs (Koyama et al., 2004). Finally, the genetics of the mass reared "Factory strain" raised in the FFPF has received little attention. As a result, the strain is highly inbred and its field performance is poor, as discussed further below (5.2).

The FFEZ was abandoned in July 2013. Government-sponsored monitoring and control measures are now concentrated in the much smaller Greater Sunraysia Pest Free Area (Fig. 1). The reasons for the abandonment of the FFEZ were the perceived lack of success of the quarantine and SIT programs, combined with reduced government willingness to support the cost of widespread monitoring programs.

4 Genetics and Genomics

Any phenotypic difference, such as the differing invasiveness of the three Australian *Bactrocera* species (Q-fly, NEO and JAR) can have many genetic (and non-genetic) components. Genome regions affecting invasiveness and mating time-of-day are expected to manifest diverse molecular mechanisms, ranging from direct effects of differences in sequence or expression, to effects downstream in a metabolic or regulatory pathway. Because of this diversity, analyses must combine a variety of approaches in sophisticated experimental designs to identify genome regions associated with the key traits (Nadeau and Jiggins, 2010).

One of the key credentials of these three species as models for studying the genetics of pest status, invasions and invasiveness, is their amenability to classical genetic experiments in the laboratory. Critically this includes not only within-species crosses, but also the fact that the three species can all be inter-crossed in the laboratory to produce hybrids which themselves produce fertile progeny, either as F_2s or beyond, or as backcrosses in either direction (Lewontin and Birch, 1966; Cruickshank et al., 2001; Shearman et al., 2010; Gilchrist et al., 2014). In combination with emerging genome sequence data (below), this will allow researchers to carry out hybridisation and selection experiments to identify genome regions that carry variants associated with invasiveness traits, if these can be identified from ecological work (see 4.4 and 5.3 below).

These three species are amenable to approaches that allow researchers to search within the genomic regions, to identify and characterise the key causal genes; these approaches include genomic mapping, transcriptomics, and modern RNAi and genetic transformation technology. Given the availability of reference genomes for all three species (Gilchrist et al., 2014), a variety of comparative and population genomic studies can also now be undertaken to understand the evolutionary and demographic processes operating on the traits in question. Below we provide a brief status report on the new genetic and genomic areas of this work, principally discussing Q-fly and NEO, which are ideal subjects because of their similarity in most aspects, other than invasiveness. However, most of what follows has also been applied to comparisons of Q-fly and JAR. After that, we consider some potential benefits for the SIT program and other possible control strategies. Parallel genomic work in the Mediterranean fruit fly *Ceratitis capitata*, is leading to insights into genes involved in mating behaviours and the competiveness of mass reared flies (https://www.hgsc.bcm.edu/arthropods/mediterranean-fruit-fly-genome-project, Gomulski et al., 2008; Calla et al., 2014).

4.1 Reference genomes

Q-fly and *Ceratitis capitata* now rank as the only tephritids with essentially complete assembled draft genomes (Qfly NCBI Accession JHQJ00000000; *C.capitata* NCBI Accession PRJNA168120 ID168120). A draft *de novo* genome assembly of the male Q-fly genome is 98% complete (Gilchrist et al., 2014). Interestingly, at 650–700 Mbp, it is three to four times the size of the genomes of most *Drosophila* species so far sequenced (Peterson et al., 2009; Seetharam and Stuart

2014). Unscaffolded assemblies of the genomes for NEO and JAR are also available (Gilchrist et al., 2014), while several genomes in the *B. dorsalis* complex are the target of current genome sequencing activities (Scott Geib, pers. comm., NCBI bioproject 208413; GenBank assembly accession: GCA_000789215.2; RefSeq assembly accession: GCF_000789215.1; WGS Project JFBF01).

Continuing assembly of these genomes will be facilitated by the availability of polytene chromosome and genetic maps for Q-fly (Zhao et al., 1998, 2003) and medfly (Bedo, 1987; Zacharopoulou, 1990). Polytene chromosome banding for Q-fly and NEO appear to be identical (Zhao et al., 1998).

4.2 Comparative genomics

Comparison of the genomes of NEO and Q-fly (Gilchrist et al., 2014) confirms previous assessments of very low genetic differentiation between Q-fly and NEO at individual loci (Bennett and Frommer, 1997; Morrow et al., 2000; An et al., 2002; Zhao et al., 2003; An et al., 2004; Raphael et al., 2004). The coding regions of the two genomes are extremely similar – much more closely related than are any *Drosophila* species pairs so far published. The synonymous substitution rate between Q-fly and NEO is only 0.21%, comparable to variation between strains of *D. melanogaster* (Zhu et al., 2012). Non-coding regions are also very similar, although NEO shows a relatively large number of deletions in non-coding regions compared to Q-fly (Gilchrist et al., 2014). The similarity of the genomes is consistent with the earlier microsatellite data, which had also revealed a level of differentiation between Q-fly and NEO comparable to that usually seen amongst populations of other species (Kinnear et al., 1998; Wang et al., 2003; Gilchrist and Ling, 2006; Zhang and Emery, 2012). However the genome data also reveal some small regions of chromosomes which show greater differentiation between Q-fly and NEO – these might be islands of differentiation associated with whatever ecological differences might exist between the two species (Via, 2012). Interestingly, intraspecific variation between Q-fly ribosomal DNA sequence reads is almost twice that found in the NEO data (Gilchrist et al., 2014), possibly due to concerted evolution (Ohta, 2000) being less effective in homogenising paralogous copies over the wider geographic scale of Q-fly populations.

Relative to the Q-fly versus NEO comparison, genomic differentiation between Q-fly and JAR is somewhat greater, being comparable to the most closely related *Drosophila* species pair, which however do not have the complete reciprocal interfertility that Q-fly and JAR display. *D. simulans* females crossed to *D. sechellia* males produce fertile females but sterile males, however, the reciprocal cross produces no viable offspring (Lachaise et al., 1986; Coyne and Orr, 1989). In contrast, laboratory crosses between Q-fly and JAR in either direction result in viable and fertile offspring of both sexes (Gilchrist et al., 2014).

4.3 Population genomics

No genome-scale population/resequencing data have yet been published for any of the three species but earlier microsatellite data on Q-fly populations (Yu et al., 2001; Sved et al., 2003; Gilchrist et al., 2006; Gilchrist and Meats, 2010) provide some intriguing pointers to demographic processes associated with some of the recent range expansions and local outbreaks of this species. Microsatellites are homogeneous over large distances in the likely ancestral home of the species in north-eastern Australia (Queensland, Fig. 1A) (Yu et al., 2001). However microsatellites diverge in presumptively more recently established populations that are now endemic further to the south (New South Wales) and to the west (Northern Territory, Western Australia). Moreover, transient genetic differentiation in the FFEZ suggests that it is possible that local outbreaks in the FFEZ in the south might derive from small populations overwintering in or near the invaded area, that are sometimes genetically distinguishable from one another or the original range (Gilchrist and Meats, 2010).

Shallow pass genome-scale resequencing is becoming an affordable tool for population genomics (Zhu et al., 2012), and its application to Q-fly and NEO, together with modern coalescence-based modelling of the data (Nadachowska-Brzyska et al., 2013) could not only provide much greater insight into the demographic histories of the two species but also identify regions of the genome subject to different selective processes in each species (Clark and Messer, 2015). Genomic data could also be used to test an intriguing hypothesis first raised by Lewontin and Birch (1966): that the invasiveness of Q-fly results from rare introgression of particular NEO genetic material into some wild Q-fly lineages. The microsatellite data provide no evidence of hybridisation between Q-fly and NEO in the wild (Gilchrist and Ling, 2006) but microsatellites do not provide the comprehensive coverage of the genome, required to test hypotheses such as this.

4.4 Quantitative genomics

Given the available genome information, previous crossing experiments in these species can now be ex-

tended to map genomic regions (Quantitative Trait Loci, or QTLs) as small as ~100 kb, which contribute to the key phenotypes. As noted, viable hybrid and backcross lines can be readily generated from forced matings amongst any two of the three species (Lewontin and Birch 1966; Cruickshank et al., 2001; Shearman et al., 2010; Gilchrist et al., 2014), opening the way for manipulative crosses to map genome regions associated with differences such as invasiveness, or the pre-mating isolation based on mating time-of-day. Early Q-fly and NEO crossing work predating the genomics era showed that the dusk mating phenotype is dominant over day mating in the F1, with backcrossing to NEO or strong selection required to recover the day mating (Smith, 1979; Meats et al., 2003b; Pike et al., 2003).

As well as identifying genomic regions relevant to invasiveness, QTL mapping can estimate those regions' size and the nature of their contribution to the phenotypes. However there may be several genes and regulatory elements within any 100kb segment of the genome, so such QTL mapping can only identify candidates for the particular genes or regulatory elements causally contributing to the phenotype. Further functional genomic work is then required to identify the latter.

Critically, QTL mapping (and SIT strain improvement, see 5.2 below) depend upon targeting the appropriate phenotypic characteristics that enhance invasiveness, but as described above, these have so far proved elusive (see 2 and 3 above, and Clarke et al., 2011), so further ecological and physiological work is required. Again, functional genomics may help here.

4.5 Functional genomics

Both of the major tools routinely used for functional genomic studies of model organisms - transcriptomics and (transient and germ-line) genetic transformation – have been developed for Australian *Bactrocera* species. Morrow et al. (2014c) have assembled quantitative transcriptomes for sexed early embryos of JAR and have used these to identify early transcripts that differ between the sexes. Stage-specific transcriptomes have been assembled for all three species (Shearman and Gilchrist, unpublished; Morrow et al., 2014a), and these have already been used to facilitate genome assemblies and annotation (Gilchrist et al., 2014). Additionally Raphael et al. (unpublished data) have recently completed quantitative time-of-day transcriptomes for brain and antenna of Q-fly and NEO, identifying genes differentially expressed between tissues, species and times of day. Moreover, as an illustration of the potential of the transcriptomic approach to identify specific candidate genes within QTL regions, the bactroceran orthologue of the *Drosophila cry* gene (which controls circadian rhythms: Emery et al., 1998; Konopka and Benzer, 1971) is up-regulated in NEO and in day-mating Q-fly/NEO hybrids, when compared to Q-fly and dusk-mating hybrids (An et al., 2004).

Transformation technologies have been developed for Australian *Bactrocera* species. Shearman has achieved transient gene-specific knock-down in Q-fly using RNAi (Raphael et al., 2014). Significantly for the SIT program (see 5.2 below), she demonstrated this with the sex-determining *tra* gene, switching development from the female to male pathway. Microinjection protocols have also been established for the stable, germ-line transformation of Q-fly using the *piggyBac* vector and two fluorescent marker genes (Raphael et al., 2011, 2014). Application of the new CRISPR transformation technology (Bassett and Liu, 2014) should further facilitate germ-line transformation in the genus *Bactrocera.*

Functional genomics may well help us track down the functional correlates of invasiveness, by comparisons of transcriptomes of flies in constantly-occupied areas versus areas that are only occupied sporadically. A similar approach in *C.capitata* has helped identify transcripts regions that affect performance of SIT strains (5.2 below).

5 Contributions to Control

The genetic data described above, in combination with ecological and physiological data, can improve current control of bactroceran pest species, as well as enlarging our understanding of the way that invasion occurs.

5.1 Scope of control

In any control program, the options for control depend heavily upon the demographics, especially immigration, extinction and recolonization. Several aspects of the microsatellite and early genome data suggest that an SIT program carried out at appropriate scale and with an appropriate release strain could achieve at least local level eradication of Q-fly. Of particular note are indications that some outbreaks in the FFEZ might have derived from flies from nearby locations in southern Australia: some southern Q-fly populations are genetically distinct from the presumptively ancestral genotypes in Queensland (Yu et al., 2001). This suggests that if broad eradication of southern populations becomes possible, it might transpire that the northern populations would then prove less able to recolonise the south. Furthermore, broad eradication in the south might be even more like-

ly to succeed if it is shown that the production of invasive strains is extremely rare – it has been hypothesised that the invasiveness of the southern populations is due to rare introgression events from NEO (Lewontin and Birch, 1966), and this hypothesis might soon be validated or refuted by population genomics data. On the other hand, microsatellite differentiation between invasive populations and the main range can be transient, suggesting that there might be repeated immigration from the main range to infested areas (Yu et al., 2001).

5.2 Efficient production of effective SIT strains

The success of SIT programs against Q-fly will require both that the sterile males should be produced more cost effectively, and that those males should be more robust in the release environment and better able to compete with wild males for mates. A mix of classical and new genetic technologies could be crucial to both these objectives. In respect of cost effective production, other SIT programs such as the one against medfly, already have GSSs (genetic sexing strains) that not only avoid the expense of raising 50% females in the last generation before release, but also avoid the possible release of fertile females (Robinson, 2002; Zacharopulou and Franz, 2013; Isasawin et al., 2014; Zepeda-Cisneros et al., 2014). Several significant discoveries and achievements have already been made towards the construction of a male-only Q-fly strain. Some of the genes of the sex-determination pathway have now been identified and expression data obtained for them; together with the availability of germ-line transformation and genome editing, this opens the way for genetic manipulation to produce male-only broods (Morrow et al., 2014b; Raphael et al., 2014). As outlined above, proof-of-principle has already been achieved via RNAi knock down of tra gene expression to convert chromosomal females into phenotypic males. Ideally, it might become possible to create a convenient oral delivery method for RNAi that is practicable to use under factory production conditions (Singh et al., 2013). Efforts are also now underway to develop this for Q-fly (Owain Edwards et al., pers. comm.).

In respect of a more robust release strain, Gilchrist and Meats (2012, 2014) have already shown that a 4-way crossing program among different Q-fly strains can be a relatively straightforward and inexpensive way to develop an outbred mass-rearing strain. They showed that this strain performs better than the current inbred factory strain under both factory and field conditions. Further improvements should be possible as population and quantitative genomics are brought to bear on relevant fitness traits.

Another issue for Q-fly SIT, where genomic data could play a role, lies in the need for a unique genetic identifier for the released flies (Scolari et al., 2008), because misidentification of a sterile fly as a wild fly could lead to loss of market access. Unique markers could be incorporated into a Q-fly release strain via genetic transformation, or through crosses to NEO, JAR or an allopatric population of Q-fly (Shearman et al., 2010; Raphael et al., 2014). A relatively easy route from a regulatory perspective would be to introgress JAR mitochondrial DNA into the release strain, and even to change mitochondrial markers between years (Shearman et al., 2010); a simple PCR-based test would then distinguish released flies from wild Q-fly. Also, fluorescent sperm marking of Q-fly, achieved through stable germ-line transformation (Raphael et al., 2014), has potential as a tool for investigating male mating success (Scolari et al., 2008), and might also be a useful marker of released flies. However, it is crucial, for the long-term use of all such genetic markers, that only sterile flies containing the markers are released, so that these markers do not become established in field populations.

In *C. capitata*, functional genomics has identified differences between wild flies and flies reared for SIT-release, with the latter showing reduced abundance of transcripts related to visual and chemical responses, possibly explaining poor performance in the wild (Calla et al., 2014). The same approach could well assist development of robust strains of Q-fly for SIT.

5.3 Integration with non-genetic research

Further ecological and physiological work is required to identify all traits that bear upon invasive ability or fitness of SIT releases – it is likely that many traits will affect both of these. Searches for differences in desiccation-tolerance, at either high or low temperatures, have not produced clear indications that temperature might limit the range of NEO relative to Q-fly (Meats, 2006; Clarke et al., 2011). Some traits related to invasiveness might emerge from detailed investigation of genomic regions that are differentiated between NEO and Q-fly. Other relevant traits might be coded in other ways, such as epigenetically, although it should be noted that given the close ecological similarity of NEO and Q-fly, there are probably very few epigenetic differences that derive from differential environmental effects.

The gut microbiomes of native and released flies may also be relevant to their fitness and dispersal ability. Little is currently known about this possibility, but precedents from other invasive pest species suggest it could

be important (Raphael et al., 2014; Chaston et al., 2015). Work led by Riegler and Morrow has commenced to identify the microbiota from both field -caught and laboratory-reared Australian tephritids (Morrow et al., 2015). Further work may be worthwhile to investigate the unusual distribution of *Wolbachia* in Australian tephritids (Morrow et al., 2014a), with one priority being laboratory experiments to test the effects of this bacterium on reproduction and fitness. High throughput characterisation of the microbiome of flies from different environments, in combination with fitness studies, could lead to the identification of microbial isolates that are suitable for use as inoculants in rearing media.

Attempts to use improved lures to lure Q-flies into traps (Clarke et al., 2011), could benefit from a better understanding of the genetics and transcriptomics of semiochemicals. Ideally such studies would be aimed a females, because if the attractant targets males (e.g., cue-lure Drew, 1989) an extremely high kill-rate would be needed to affect population numbers in the next generation.

5.4 Species identification and invasion in the *Bactrocera*

Finally we note that from a biosecurity perspective, and in the face of climate change, it is likely to become increasingly important to distinguish and monitor other *Bactocera* species that could invade Australia's horticulture, either from northern Australia or from elsewhere in the Indo-Australian archipelago (Simpson and Srinivasan, 2014). As noted, the comparative genomic study of NEO and Q-fly, as well as providing tools to investigate the molecular genetic bases and phenotypic correlates of the invasive character, has already identified many potentially diagnostic insertion-deletion differences between the two species. Some of these differences could well provide the long sought-after diagnostic markers for Q-fly and NEO embryos and larvae. Extending the comparative genomics to other *Bactrocera* of greater or lesser pest status–would seem well worthwhile, both to provide tools for the rapid detection of emergent fruit fly threats and to better understand the genetic mechanisms of invasiveness. For extending the work to other species complexes within the *Bactrocera*, the extremely tractable model system of the more invasive Q-fly and the less invasive NEO, will allow ready identification of candidate genes amenable to further investigation of species identity, ecology, and invasiveness.

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