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SYMPOSIUM

Testing the Hamilton–Zuk Hypothesis: Past, Present, and Future

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Synopsis Hamilton and Zuk proposed a good-genes model of sexual selection in which genetic variation can be maintained when females prefer ornaments that indicate resistance to parasites. When trait expression depends on a male's resistance, the co-adaptive cycles between host resistance and parasite virulence provide a mechanism in which genetic variation for fitness is continually renewed. The model made predictions at both the intraspecific and interspecific levels. In the three decades since its publication, these predictions have been theoretically examined in models of varying complexity, and empirically tested across many vertebrate and invertebrate taxa. Despite such prolonged interest, however, it has turned out to be extremely difficult to empirically demonstrate the process described, in part because we have not been able to test the underlying mechanisms that would unequivocally identify how parasites act as mediators of sexual selection. Here, we discuss how the use of high-throughput sequencing datasets available from modern genomic approaches might improve our ability to test this model. We expect that important contributions will come through the ability to identify and quantify the suite of parasites likely to influence the evolution of hosts' resistance, to confidently reconstruct phylogenies of both host and parasite taxa, and, perhaps most exciting, to detect generational cycles of heritable variants in populations of hosts and parasites. Integrative approaches, building on systems undergoing parasite-mediated selection with genomic resources already available, will be particularly useful in moving toward robust tests of this hypothesis. We finish by presenting case studies of well-studied host–parasite relationships that represent promising avenues for future research.

Introduction

Differences in fitness are central to evolution. Actually measuring fitness, however, is a tricky proposition, since it requires assessing the degree to which genes are transferred to subsequent generations. Scientists, particularly those studying organisms in the field, often find it easier to use proxies such as condition or vigor, with the assumption that individuals in better condition will also exhibit higher fitness. Here, too, we are dealing with slippery concepts. What exactly do we mean by terms such as "condition," "quality," or "vigor"?

Nowhere is this difficulty more apparent than in studies of sexual selection, where ornamental traits often are suggested to be condition-dependent, meaning that their expression depends at least in part on environmental factors such as nutrition, social interactions, and disease. For example, male house finches (*Haemorhous mexicanus*) with redder plumage can advertise their ability to acquire carotenoid pigments via their diets; these pigments are required for the production of red feathers (Hill 1992; Hill et al. 2002). Female house finches that choose such males as mates therefore obtain the genes associated with that ability for their offspring (Hill 1991), but how do we determine whether an individual is in good condition? Do any universal indicators of quality exist?

One solution to this problem was proposed by Hamilton and Zuk (1982), who suggested that health and resistance to parasites were indicators of condition that could drive the evolution of secondary sexual characteristics such as colorful plumage. The genes for resistance would change over time as the pathogens evolved counter-defenses to them, thus generating continual heritable variation in fitness. Females would always use the same indicators, namely the degree of development of ornaments,

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and they would always indicate overall health, but the genes associated with preferred ornaments would vary.

This hypothesis spurred a large number of tests in a variety of taxa, with attempts to determine whether ornaments were linked to resistance to parasites, whether females used such ornaments as a basis for mate choice, and whether parasites' and hosts' genotypes co-varied in the predicted manner (Zuk 1992; Møller et al. 1999). Although intuitively appealing, this approach often has led to ambiguous results; if no relationship between mate choice and parasite burden was found, one could always argue, for example, that the particular parasite chosen for testing is not the appropriate one. In recent years, therefore, researchers have gauged resistance by measuring immunity directly, rather than by examining parasite burdens (Sheldon and Verhulst 1996; Roberts et al. 2004; Lawniczak et al. 2007).

Here, we aim to integrate Hamilton and Zuk's (1982) original verbal model and the subsequent tests of its predictions with the current revolution in genomics. We begin by briefly exploring the importance and potential implications of Hamilton and Zuk's (1982) hypothesis. We then review the predictions made by Hamilton and Zuk and discuss some of the limitations in testing these predictions and interpreting the results of studies based around them. Fundamentally, Hamilton and Zuk's hypothesis relies on the assumption that change in host and parasite genotypic frequencies over time will conform to a "permanently dynamical" scenario like that exhibited by stable predator–prey limit cycles (May 1972). We argue that crucial evidence pertaining to such cyclical co-adaptation of hosts' and parasites' genotypes is sorely lacking. The past decade, however, has seen a revolution in the amount of sequence-based data one can acquire for virtually any biological system. We, therefore, ask whether, and to what extent, longitudinal, high-throughput genomic data could provide us with the mechanistic details that would finally allow for rigorous tests of the hypothesis. In particular, the greater accessibility of genome-scale sequencing leads us to ask whether genomics data can help us detect the process described by Hamilton and Zuk (1982). We present brief reviews of several host–parasite systems that we think are most amenable to genomics-enabled study of the Hamilton and Zuk hypothesis.

Parasites and the lek paradox

In the late 1970s, the question of how additive genetic variation could be maintained in traits under sexual selection was receiving increased attention (Borgia 1979). When females choose mates based on arbitrary ornamental traits and receive genetic benefits for their offspring as a result, one would predict that the heritable genetic variation in fitness should be lost over time, leading to an eventual lack of benefit to females for being choosy. Although this scenario applies to many mating systems with strong directional selection, it came to be known as the "lek paradox" due to the extreme skew in mating success among males breeding in leks (Borgia 1979).

During this same period, multiple books and papers discussing the importance of parasites to hosts' ecology and evolution were also produced (Anderson and May 1978, 1979; May and Anderson 1978, 1979; Price 1980). It was finally becoming clear that parasites, broadly defined, should be considered as important factors affecting and even driving the evolution of their hosts. Hamilton and Zuk (1982) recognized that parasites could provide a potential solution to the loss of genetic variation predicted by the lek paradox. Specifically, Hamilton and Zuk (1982) argued that additive genetic variance in fitness could be maintained when the traits under sexual selection are associated with genes for resistance to disease. A host's resistance genes will cycle with the parasite's virulence genes, and thus the genetic variation associated with sexually selected traits would come from host–parasite co-evolution. Hamilton and Zuk (1982) proposed that specialist parasites should be more likely to produce co-evolutionary cycles since the feedback from any one host species to a generalist parasite's gene pool would be small. This issue remains unclear, however, since even in the original study the authors found evidence for a relationship between ornamentation and *Toxoplasma*, an extreme generalist blood parasite.

This hypothesis has deservedly been credited with early promotion of the idea that parasites and health are important factors in the host's reproduction and behavior. Subsequently, several hypotheses have been proposed that use similar reasoning, typically focusing instead on immunity of the host as a quantifiable life-history trait, rather than on the measurement of parasites *per se*. For example, Folstad and Karter (1992) proposed the influential immunocompetence-handicap hypothesis, arguing that the suppressive effects of testosterone on the immune system result only in the best males having the ability to develop attractive ornaments and to fight disease. Thus, rather than measuring ornamentation and parasite load, many researchers began measuring ornamentation, testosterone, and general immune

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function. A hypothesis that invokes a role for testosterone, however, cannot be applied to invertebrates, and thus has limited explanatory power for the evolution of sexually selected traits (Rolff 2002; Rolff and Siva-Jothy 2003). Hypotheses that do not invoke testosterone in mediating the relationship between ornamentation, reproduction, and immunity therefore have also been proposed (Rolff 2002). The field of ecoimmunology has also become prominent (Sheldon and Verhulst 1996; Demas and Nelson 2012). Other hypotheses following Hamilton and Zuk (1982) include the parasite-avoidance hypothesis (Borgia 1986) and the contagion-indicator hypothesis (Able 1996).

Predictions and limitations

Hamilton and Zuk's (1982) model proposes that parasites drive the evolution of ornaments that signify resistance to disease. Essentially, the greater the selective pressure by parasites, the better it will be for females to base their choice of mate on a trait that honestly indicates a male's susceptibility or resistance to local parasites. At the interspecific level, this will translate into a positive association between ornamentation (e.g., brightness, song complexity, and energetic courtship display) and parasitization (Fig 1A).

The interspecific prediction has been tested repeatedly over the past 30 years (Hamilton and Zuk 1982; Read 1988; Read and Weary 1990; Møller 1990, Møller et al. 1999; Hamilton and Poulin 1997; Garamszegi and Møller 2012). Unfortunately, testing has proven more intractable than originally imagined, largely due to a variety of unavoidable methodological limitations (Zuk 1992; Garamszegi and Møller 2012). Briefly, a lack of consistency in the methods of collecting data across studies has made it difficult to pool results for meta-analysis. For example, researchers have not always measured the same parasite(s), in the same way, at the same time of year, and/or at the same life-history stage. For many years, a major hurdle was the lack of objective methods for measuring ornamentation, but this has largely been resolved, at least when it comes to brightness and coloration, due to the widespread availability of spectrophotometers and software for extracting relevant data (Montgomerie 2006) and visual models based on the perception of the receiver (Vorobyev et al. 1998). Perhaps the availability of objective measures of reflectance inadvertently perpetuated another problem with tests of the interspecific hypothesis; the fact that it has so far primarily been tested on birds. Although birds are convenient models for sexual selection because they, like humans, tend to rely on visual signals, it would be better to utilize a broader range of taxa. From a comparative perspective, it is likely that organisms with different life histories will resolve host–parasite relationships in various ways; for example, components of the avian adaptive immune system may in fact be co-evolving with parasites, whereas invertebrates, with no adaptive immunity, may rely on other systems. From a practical perspective, birds live a relatively long time, and so we are likely to gain more cross-generational information by studying organisms with fast generation times. Finally, the inability of early studies to correct for non-independence of traits due to shared ancestry has shown major improvement over the years due to development of a comparative method and more refined phylogenies in many taxa (Felsenstein 1985; Harvey and Pagel 1991). Not only is it now possible to correct for the non-independence of ornamentation and other traits across species of interest, but it is possible to do so based on well-supported trees built

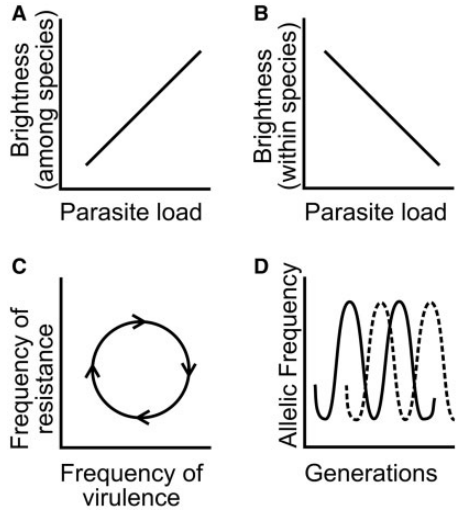

Fig. 1 Predictions of the Hamilton–Zuk hypothesis of parasite-mediated sexual selection (**A** and **B**). (A) At the interspecific level, those species with the lowest parasite loads will be subject to the weakest amount of sexual selection, whereas those that are subjected to high levels of parasites will be subject to the strongest levels of sexual selection and thus evolve the brightest or most ornamented traits. (B) At the intraspecific level, the brightest, most ornamented males will have the lowest parasite loads. (**C**) These predictions were originally made in light of contemporary gene-for-gene models of plant–pathogen co-evolution that require stable limit cycles between the frequency of resistance alleles in the host's population and the frequency of virulence alleles in the parasite's population (adapted from Leonard 1993). (**D**) Subsequent matching-alleles models incorporating invertebrate self/non-self recognition mechanisms have demonstrated the feasibility of stable negative frequency-dependent cycling between the host's resistance allele(s) (solid line) and the parasite's virulence allele(s) (dashed line).

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from large numbers of genes (Omland and Hofmann 2006).

Most recently, Garamszegi and Møller (2012) have revisited the interspecific predictions of Hamilton and Zuk (1982), and have examined the relationship between blood parasites (*Haemoproteus*, *Plasmodium*, *Trypanosoma*, and *Leucocytozoon*) and sexually selected traits for nearly 200 and over 450 species of birds for which they obtained data on song and on plumage color, respectively. Garamszegi and Møller's (2012) study incorporated many advances in testing the interspecific predictions, notably by using better methods for the quantification of ornaments than did earlier papers, by using better methods of detecting parasites, and by correcting for shared ancestry. Even with many methodological advances and such large sample sizes, however, the results remain somewhat ambiguous. Although many comparisons had only very weak to moderate effect sizes, several did "reach levels that were evolutionarily meaningful" and provided some support for the prediction. However, high levels of intraspecific variation were shown to confound the testing of the interspecific prediction.

That intraspecific variation would confound tests at the interspecific level should not be too surprising, given that variation among individuals is also expected to occur when Hamilton and Zuk's mechanism is at work. Hamilton and Zuk also predicted a negative relationship between ornamentation and parasitism within populations or species (Fig. 1B). Individuals with greater ornamentation should be more resistant to parasites and thus be capable of producing more attractive display traits. Those that are riddled with parasites will not be capable of producing an attractive ornament and will appear dull or produce less complex songs or displays.

Many of the issues described for tests of the interspecific prediction also apply to the intraspecific prediction. Which parasite and ornament are the best to study in a given system? At what time of year/stage of development should they be measured? What methods are best for measuring the presence or abundance of the parasite and assessing the host's ornament? How can one disentangle resistance and exposure in the wild—if an individual has good ornaments but few parasites, is that because that individual is really resistant or just because it has not been exposed? A further crucial issue confounds our understanding of the data: a lack of relationship between ornamentation and parasite load is virtually uninterpretable with respect to the hypothesis. This is in part due to the questions listed above, but also because of the dynamic nature of the mechanism underlying the predictions in the first place, that is, co-evolutionary cycles between the host's alleles for resistance and the pathogen's alleles for virulence (Fig. 1C).

Models of co-evolutionary cycles

The model as first proposed by Hamilton and Zuk was influenced by the gene-for-gene model of plant–pathogen interactions, in which a parasite's genotype can be either avirulent or universally virulent (Flor 1971; Thompson and Burdon 1992). Since then, models of matching-alleles have been developed to better represent the infection-dynamics of invertebrate self/non-self recognition systems (Fig. 1D). Matching-alleles models require a specific genetic match between the host and the parasite for infection to take place, after which genetic polymorphism can be maintained via negative frequency-dependent selection. Agrawal and Lively (2002) demonstrated that gene-for-gene and matching-alleles can be considered opposite ends of a spectrum, along much of which stable limit cycles between resistance and virulence can be achieved. This suggests that host–parasite interactions following a variety of scenarios can, in theory, produce co-adaptive cycles of resistance and virulence. Although much empirical evidence exists for the gene-for-gene model in plant–pathogen systems (Thompson and Burdon 1992), support for matching-alleles models was shown empirically only very recently, utilizing the *Daphnia magna–Pasteuria ramosa* system (Luijckx et al. 2013).

What remains to be done, and how do we get there?

It is now widely accepted that parasites can be important in sexual selection, as they are in other aspects of a host's ecology and evolution. Definitive evidence of the process outlined by Hamilton and Zuk (1982), however, has remained elusive. Perhaps it is time to turn from looking at correlations between ornamentation and parasite load and instead examine the underlying mechanism that would produce those patterns, that is, the stable co-evolutionary cycles between the host's resistance and the parasite's virulence. Genetic/genomic investigations of hosts and parasites have identified the types of allele-matching and frequency-dependent allelic cycles predicted by Hamilton and Zuk (Eizaguirre et al. 2012; Luijckx et al. 2013). As yet, however, these have not been shown to be related to the expression of ornamental traits. As with traditional correlational studies of ornament quality-parasite load, detection of co-evolutionary cycles in natural

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populations is likely to be more difficult than in controlled settings due to variation in individual host's exposure to different parasite levels and communities. Currently, the most promising approaches likely will combine genomics data with time-shift (see below) sampling and/or multi-generational study designs when possible, and use well-studied host–parasite systems known or expected to be undergoing negative frequency-dependent selection. Below we provide basic descriptions of several genomics approaches particularly well-suited to identifying limit cycles at the molecular level, followed by a brief explanation of some practical considerations for the, as yet, uninitiated.

High-throughput genetic data: options in breadth and depth

The past 5–7 years have given us decreased costs and enormous technological advancements resulting in massive amounts of sequence data, as well as an ever-growing pile of empirical studies, reviews, and opinion articles foretelling the brave new world we are embarking upon as we ride the crest of the wave of the "omics" tsunami. Methods for obtaining high-throughput genetic data have rapidly been adapted and refined from early shotgun sampling approaches so that now various scales of coverage can be targeted (Head et al. 2014). Some examples of coverage targets include full genomes, metagenomes, and transcriptomes, which trade-off depth of coverage (and confidence in the ability to assign genotypes) with breadth of sequence information (i.e., proportion of the entire genome sequenced). Other "reduced-representation" methods allow for the selective capture of small, specific bits and pieces of the genome (Table 1) and provide the opportunity for greater depth of coverage at the expense of breadth. In sum, most researchers can target how much and which portion(s) of the genome they require, depending on the specific question at hand.

Sequencing and assembly of non-model eukaryotic genomes are becoming ever more commonplace (Ellegren 2014). Genome sequencing obviously has facilitated, and continues to facilitate, our understanding of the organization of the genome, but it also provides a backbone or scaffold upon which one can map the whole genome or subsets of the genome from additional individuals (Ellegren 2014). Studies combining a reference genome with either whole genomes (called genome resequencing) or portions of genomes from multiple individuals and/or populations offer incredibly powerful designs for researchers interested in, for instance, functional genomics, phylogeography, or mapping associations of traits (Davey et al. 2011; Ellegren 2014).

An important subset of the genome being targeted by many researchers is the transcriptome, the portion of genetic material transcribed into RNA. Much of the transcriptome represents the protein-coding regions of the genome and thus can be studied to identify candidate genes underlying phenotypic variation. Experiments designed to identify differential gene expression across the transcriptome are valuable for identification of functionally important genes under conditions of interest. Until recently, these studies were primarily conducted using microarrays, a hybridization-based method capable of providing relative expression levels at hundreds of thousands of loci at once (Malone and Oliver 2011). Microarray data often are cheaper to obtain and easier to analyze than are gene expression data produced by high-throughput sequencing (often referred to as RNAseq), especially when a reference genome or Expressed Sequence Tag library already exists to facilitate construction of the reference microarray slide (Malone and Oliver 2011). Furthermore, many studies have validated the similarity in gene expression when comparing the two methods (Malone and Oliver 2011; Guo et al. 2013). The major advantages of RNAseq approaches over microarrays are that (1) RNAseq is quantitative whereas microarray data are relative and (2) RNAseq also produces the sequence of the transcriptome (Wang et al. 2009). This information may identify coding mutations or variants that underlie, or are associated with, phenotypic differences (De Wit et al. 2012).

A variety of additional reduced-representation methods allow for the targeting of specific regions, characteristics, or loci in the genome (Table 1). Reduced-representation methods simply refer to those that target a particular subset of the genome, for example, only the coding regions of genes (exomes) or only genomic locations containing a specific restriction-enzyme cutting site (RADseq). Such targeting increases the depth of coverage at those locations. These currently fall into two main categories: sequence-capture and restriction-enzyme methods. When targeting a reduced portion of the genome, it is commonplace to attach barcodes or linker sequences to the nucleic acids from individual samples, allowing for the potential to multiplex hundreds of individuals in a single sequencing run. The number of individuals that should be barcoded and subsequently pooled together is largely limited to the breadth of sequencing required by the method (Table 1). Barcoded samples can then be separated from one another bioinformatically after sequencing

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Table 1 Options in breadth and depth of high-throughput genetic data

Scale	Output	Multiplexed Individuals?	Relative Depth of Coverage	Usefulness to Testing H–Z	Relative Cost
Genome	Much of the genome	No	Low to high	Reference backbone	High
Metagenome	Parasite sequences	No	Low	Identification of parasites	High
Reduced-representation methods					
Transcriptome	Expressed genes	Yes—few	Moderate	Identification of candidate genes	Moderate
Restriction-enzyme associated	Genome-wide SNPs	Yes—dozens to hundreds	Very high	SNP associations with ornament/resistance/virulence	Low to moderate (depending on the number of individuals genotyped)
Sequence-capture probes	Varies depending on target	Yes—varies depending on target size	Moderate to high	Identification of genes underlying SNP associations	Moderate (depending on portion of genome to be probed)

has been performed (Elshire et al. 2011; De Wit et al. 2012).

Sequence-capture methods use complementary probes designed to selectively bind to the region(s) of interest, thus "capturing" the targeted portion of the genome prior to sequencing. These approaches often (but not always) require knowledge of at least some portion of the genome or transcriptome. Examples of common sequence-capture targets include exomes, a specific chromosome, the major histocompatibility complex (MHC), and highly repetitive regions (microsatellites). This last example is now being commonly used for the identification of polymorphic population genetic markers and does not require any prior genomic information (Guichoux et al. 2011).

Restriction-enzyme Associated DNA sequencing (RADseq), on the other hand, uses restriction-enzyme digestion of the genome, followed by selective PCR amplification, specifically allowing one to sequence only those short regions surrounding restriction sites (Davey and Blaxter et al. 2011). With sufficient depth of coverage, a single experiment can yield genotypes of thousands of variable loci for dozens or hundreds of individuals. Depending on the length of each fragment sequenced, RAD markers can be mapped onto a genome reference and/or primers can be designed for further testing of specific loci (Davey et al. 2011). Such information from both host and parasite should allow for the isolation of genomic regions associated with resistance and virulence.

Metagenomics represents the special case of a sequencing approach that, when applied to a host–parasite relationship, one could use to intentionally remove the host's genomic information and specifically target only the sequencing of parasites (Allen and Banfield 2005; Kurokawa et al. 2007). The goal of metagenomics is not to provide a perfect map of the genetic material for a single organism, but rather to provide some genetic information for many species contained within a single sample. Metagenomics may aid the testing of the Hamilton–Zuk hypothesis by identifying most, or all, possible parasites which could be co-evolving with the host species via limit cycles. Such an approach would certainly require extensive experimental follow-up work to determine the role of novel microbes in the host; thus, the current usefulness of this technique in identifying appropriate parasites is largely speculative.

Hamilton and Zuk meet genomics

Perhaps the two most promising high-throughput sequencing approaches for identifying candidate loci or regions of the genome involved in parasite-mediated sexual selection are (1) genome resequencing and (2) genome-wide, marker-based approaches (like RADseq). In a genome resequencing study, one might select a subset of host individuals representing the most and least resistant (or the brightest and dullest) in the population, as well as a subset of parasite individuals representing the most and least virulent in the population. Comparisons of the genomes of individuals with these opposing phenotypes can allow for the isolation of genomic regions and specific genes or variants responsible for such differences (Shapiro et al. 2013).

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Pursuing genotype by sequencing approaches like RADseq, on the other hand, will require a trade-off between a reduction in the coverage of the genome and an increase in the number of individuals that can be sampled in a single experiment. Of course, it is possible with a RADseq approach to miss regions of the genome exhibiting signatures of co-evolution because of incomplete coverage. However, if markers associated with resistance and virulence can be identified and mapped to hosts' and parasites' reference genomes, it will also be possible to identify the genes in those regions as possible candidates. However, validation of the Hamilton–Zuk hypothesis does not require identifying the underlying functional genes, even if we might be interested in knowing what they are. The major benefit to using RADseq for isolating the underlying genetics of a dynamic process like that described by Hamilton and Zuk (1982) is that these experiments can identify polymorphic markers throughout the genome that are likely to be of interest, while simultaneously providing individual genotypes. Thus, the identification of markers and the collection of data are essentially combined. Once the markers or genes are identified, future genotyping of host and pathogen can be performed without the need for genomic-scale sequencing.

Simply identifying the genes likely to be responsible for resistance and virulence, however, will not provide the essential information needed to identify stable limit cycles. Follow-up work will be required to determine the frequency of these alleles in current and future generations to test whether over time their dynamics fit Hamilton and Zuk's (1982) predictions. There are generally two feasible approaches to identifying limit cycles when long generation times limit the number of generations one can follow in real time: geographic comparisons and time-shift experiments, which "test the performance of one antagonist population from a moment in time against the other population from the same and different moments in time" (Gaba and Ebert 2009).

Simultaneous comparisons of genotype frequencies of spatially distinct populations have the potential to reveal the type of co-evolutionary dynamics present in a given host–parasite pairing and determine whether they conform to our expectations of stable limit cycles. When possible, however, time-shift experiments would allow for much more straightforward predictions and interpretation of results. Time-shift experiments not only allow for the study of temporal variation in hosts' and parasites' genotypes over time, but importantly allow for the testing of interactions between past, present, and future genotypes (Gaba and Ebert 2009). Time-shift experiments require that at least one, if not both, species be held in evolutionary stasis, which is the major roadblock to its implementation in studies of parasite-mediated sexual selection. However, although few animals exhibiting sexually selected traits can be held constant over time, fungal organisms like *Saccharomyces cerevisiae* use sexually selected pheromones and can be freeze-dried for long-term storage (Jackson and Hartwell 1990). Furthermore, certain types of parasites are easily frozen and reanimated upon thawing, and thus past parasites can be used to infect "future" genotypes (now the present) of their host.

Some practical considerations of genomics approaches

The relatively young field of genomics has moved quickly, but the rapid emergence and availability of high-throughput sequencing datasets have just begun to show us what we can learn about evolutionary processes in natural populations from this type of data (Rokas and Abbott 2009). For example, much discussion is currently taking place regarding how best to identify signatures of adaptation using genomic data (Nadeau and Jiggins 2010; Stapley et al. 2010; Barrett and Hoekstra 2011; Savolainen et al. 2013).

We are in an era in which the genomes of species with well-studied sexually selected ornaments are being sequenced (e.g., *Gallus gallus*, red jungle-fowl; *Gasterosteus aculeatus*, three-spined stickleback; *Taeniopygia guttata*, zebra finch; and *Anolis carolensis*, green anole) by both consortiums and individual laboratories. Utilizing species with freely available genomes, sexually selected traits, and characterized host–parasite relationships seems a promising and fruitful place to begin for those interested in testing the Hamilton–Zuk hypothesis without the resources to compile a *de novo* genome. Investigations of gene expression and/or genome-wide markers can be undertaken immediately using such systems.

The study of host–parasite co-evolutionary cycles will require knowledge of parasites' genomes as well. Although genome-sequencing has been completed for many parasites, the majority of sequenced parasites are those of importance to human health (Jex et al. 2013). Although certain types of parasite have relatively small genomes and will be trivial for most individual laboratories to sequence (phages, viruses, and certain bacteria), many parasites of interest will be eukaryotes and researchers will be confronted by similar difficulties as when sequencing a host species

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(Cantacessi et al. 2012). Finally, an even bigger (and long-standing) problem is determining which parasite(s) to target for detailed study. Fortunately, metagenomic, or community genomic, approaches provide a promising avenue for identifying and selecting those parasites most likely to be involved in co-adaptive cycles with the host of interest (Wooley and Ye 2010; Ng et al. 2011; The Human Gut Microbiome Consortium 2012; Jex et al. 2013).

Candidate host–parasite systems

We have selected three host–parasite systems to explore as good candidates for investigating co-evolutionary cycles with respect to the Hamilton–Zuk hypothesis. We chose them not only because they represent natural host–parasite systems that are tractable, both in the field and in the laboratory, but also because they represent varying degrees of genomic resources available for host and parasite species.

Three-spined stickleback—*Schistocephalus solidus*

Gasterosteus aculeatus, the three-spined stickleback, has been the subject of enormous scientific study (Bell and Foster 1994; Ostlund-Nilsson et al. 2006). A small teleost fish, it has proven amenable to an array of field-based and laboratory-based research due to its size, availability, wide distribution, and interesting variation in life-history traits, particularly with respect to reproductive traits and behaviors. More recently, it has become even more amenable to studies of functional genomics with the release of its genome sequence (http://www.ensembl.org/Gasterosteus_aculeatus/Info/Index). Due to a long history of behavioral study and an extensive list of natural parasites (Barber 2013), this small fish is an excellent vertebrate model for the study of parasite-mediated sexual selection. Male sticklebacks exhibit red carotenoid pigment-based coloration. Milinski and Bakker (1990) used laboratory mate-choice experiments to first demonstrate that female sticklebacks choose males based on the redness of their throats. Subsequent studies found that the coloration of males co-varies with infection status with a range of parasites (Folstad et al 1994) and that resistance is heritable, such that the offspring of redder males are more resistant to infection by the tapeworm *S. solidus* than are the offspring of less red males (Barber et al. 2001). Interestingly, the carotenoid pigments required for the red coloration and related to infection by *S. solidus* are acquired by sticklebacks through their diet of copepods. These studies provide convincing support that male sticklebacks could be indicating parasite resistance alleles via their coloration. That both host and parasite can be reared and manipulated in the laboratory make study of this system even more promising (Barber and Scharsack 2010; Barber 2013).

Polymorphisms in the MHC, a component of vertebrates' adaptive immune system, have been examined in three-spined sticklebacks with respect to parasite load both in the field and in the laboratory (Eizaguirre et al. 2011, 2012). Although not specifically targeted at the relationship with *S. solidus*, these studies demonstrated that MHC alleles varied according to habitat (e.g., river versus lake) which corresponded to the differences in parasite communities located in those habitats (Eizaguirre et al. 2011). Furthermore, the authors showed that by varying the exposure of host sticklebacks to different parasites in the laboratory, they could induce rapid changes in the frequency of adaptive MHC resistance haplotypes; after only two generations of selection, individuals were more resistant to parasitic challenge than were their forbears (Eizaguirre et al. 2012).

Considering the fact that many full-genome sequences of three-spined sticklebacks now exist (e.g., Jones et al. 2012) and that RADseq technology has most thoroughly been developed by researchers studying this species (Baird et al. 2008; Hohenlohe et al. 2010), identifying three-spined sticklebacks' allelic variants utilizing genome-wide markers should not present much difficulty. For example, Hohenlohe et al. (2010) identified over 45,000 Single Nucleotide Polymorphisms (SNPs) for use in their study of parallel adaptation in oceanic and freshwater populations. Furthermore, sticklebacks' MHC-region-specific sequence-capture probes could be used to specifically test for frequency-dependent selection at parasite-specific resistance alleles. The major hurdle in monitoring host–parasite genetic co-evolutionary cycles in this system will be the genome sequencing of a well-suited parasite, that is, *S. solidus*.

Drosophila melanogaster—*Asobara tabida*

Invertebrate organisms represent potentially excellent models for the study of the Hamilton–Zuk hypothesis. Although most studies to date have used vertebrate taxa (especially birds, but also fish, reptiles, and amphibians) (Møller et al. 1999), invertebrates have many advantages. For example, their lack of complex adaptive immunity suggests that it may be easier to identify the molecular mechanisms responsible for resistance in invertebrates (Rolff and Siva-Jothy

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2003; Adamo 2012). Additionally, certain invertebrate species or populations will not only fit the requirements for studying the Hamilton–Zuk hypothesis (i.e., the existence of sexually selected traits and the presence of co-evolving parasites), but also have comparatively short lifespans which allow for generation of lines selected for resistance or susceptibility to parasites, on top of having a wealth of genomics resources available (www.flybase.org). This final specification, of course, points directly at drosophilids, with the genomes of 12 species sequenced and publicly available, as suitable models for study.

In the first attempt to use artificial selection to identify genetic correlations between the resistance to a natural parasite and the host's mating success, Rolff and Kraaijeveld (2003) used multiple lines of *D. melanogaster* selected for resistance against infection by the koinobiont parasitoid wasp *A. tabida*. In comparison with control lines, males from resistant lines were more successful at obtaining matings than were controls, and they obtained matings more quickly. Although this study provided some support for a Hamilton–Zuk mechanism, the authors acknowledged several alternative explanations for the relationships. Unfortunately, this study did not examine ornamentation (singing, courtship, and cuticular hydrocarbons) in the flies with respect to resistance and mating success which could have provided stronger support for the Hamilton and Zuk hypothesis.

More recently, researchers have begun exploring the transcriptional responses of *D. melanogaster* to actual infection with *A. tabida* and to artificial selection for resistance to *A. tabida* infection using microarray hybridizations (Wertheim et al. 2005, 2011). Somewhat unexpectedly, the lists of genes identified by these two studies were mostly non-overlapping, suggesting that the genes plastically responding to infestation in normal fly lines (controls) are not the same as those that are under selection for resistance (Kraaijeveld and Godfray 2009; Wertheim et al. 2011). These results demonstrate that simply identifying the genes that respond to infection by altering gene expression may not actually isolate the targets of selection for resistance in certain systems.

Interestingly, most of the genes responding to parasitoid infestation had not previously been identified as having immune function (Wertheim et al. 2005), thus focusing solely on candidate genes with known relevant function can be misleading and overly simplistic. Herein lies the power of genome-wide approaches—the ability to identify candidates for resistance without automatically excluding genes with previously unknown immune or disease-resistance function. In this system, in fact, the most likely candidates at this point are genes never before identified as being involved in immunity or in defense against parasitoids (Wertheim et al. 2011).

House finch—*Mycoplasma gallisepticum*

Female house finches prefer to mate with red males (Hill 1990, 1991), and such males provide more food to their incubating mates and to young in the nest (Hill 1991, but see also Duckworth et al. 2003). Male house finches' coloration is affected by conditions during molt, including access to carotenoid pigments (Hill 1992, Hill et al. 2002), general nutrition (Hill 2000), and parasite load (Brawner et al. 2000; Hill et al. 2004). Some wild populations of house finches are host to *M. gallisepticum* (MG), a bacterial pathogen first identified in finch populations in 1994 (Ley et al. 1996). Owing to its relatively small genome (~1 Mb) and its importance as a pathogen of poultry, the MG genome was sequenced over a decade ago (Papazisi et al 2003). More recently, the genomes of multiple isolates cultured from wild-caught, infected house finches at various times, and from various populations have been sequenced (Delaney et al. 2012; Tulman et al. 2012). Adding to its utility, this pathogen is easily stored long-term in frozen culture. Thus, it is possible to use combinations of historical and modern samples of MG isolates for experiments involving controlled infections of present-day birds (Hawley et al. 2013), thereby representing a "host fixed-parasite changing" time-shift experimental design (Gaba and Ebert 2009).

During molt, male House Finches infected with MG grow feathers that are significantly less saturated and less red (i.e., more yellow) than do males that are not infected with MG (Brawner et al. 2000; Hill et al. 2004). Furthermore, red males successfully clear symptoms of MG infection faster than do yellow males (Hill and Farmer 2005). These studies suggest that feather color indicates a male's infection status during molt as well as his ability to fight infection.

Importantly, populations from the eastern and western halves of the United States have different co-evolutionary histories with this pathogen; with the disease spreading to most western populations in only the past few years, whereas eastern populations have been subjected to it for ~20 years. Using microarrays, Bonneaud et al. (2011) showed that these differences between eastern and western populations of finches in their exposure and opportunity to co-evolve with the parasite resulted in rapid evolution of differences in resistance to experimental

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laboratory infection with MG. Since then, an RNAseq dataset of the house finch's spleen transcriptome has been published (Backström et al. 2013). The ability to combine these powerful genetic and genomic tools with time-shift experiments in which the host is fixed and the parasite is changing make the house finch–MG relationship a promising avian system for pursuing the resistance–virulence cycles described by Hamilton and Zuk (1982).

Conclusions

Hamilton and Zuk (1982) has been one of the most influential "good-genes" hypotheses of sexual selection largely because it was the first to propose a solution to the lek paradox in which genetic variance associated with fitness is depleted until female choice is no longer beneficial. As has been repeatedly pointed out, however, the hypothesis suffers both from a lack of direct tests and from ambiguous results arising from indirect correlative evidence (Zuk 1992; Møller et al. 1999). On the other hand, it has had a long arm of influence, leading others to recognize that ornamental traits might not only function as indicators of immune quality or individual health, but also that the variability inherent in the immune system is a good target for the maintenance of both additive and non-additive types of genetic variance that need to be associated with good-genes models of sexual selection in order for them to work. In addition, it was one of multiple pivotal papers to come out at that time stressing the importance of parasites as environmental factors that are intrinsic to virtually all life on the planet, and thus must be included in our considerations and understandings of evolutionary processes (Anderson and May 1978, 1979; May and Anderson 1978, 1979; Price 1980).

The emergence and availability of 'omics datasets has only begun scratching the surface of what we can learn about molecular evolution and the role that molecular dynamics and interactions play in influencing the rate and direction of evolution, not to mention their role in constraining evolution. In many cases, identification of the underlying molecular genetic mechanisms, the "black box", is aiding, and will aid, in understanding the influence of ecology and genetics on the evolution of phenotypes of interest, for example, color polymorphism in the beach mouse (Hoekstra et al. 2006) and pelvic girdle and reduction of armor in sticklebacks (Shapiro et al. 2004; Colosimo et al. 2004). Continued development of bioinformatics tools for dealing with, and making sense of, high-throughput genomic data has made it easier to handle and describe, but in many cases making evolutionary sense of the data is still a complex matter. In many ways, we are in a new era of natural history, involving computational algorithms and digital repositories rather than calipers, collecting jars, and field observation. Functional predictions of genomic variants are difficult to make for any organism. This is particularly problematic for studies of organisms that only a few years ago had a few microsatellite genetic markers and maybe a mitochondrial sequence, but now have quantitative data across transcriptomes of multiple tissues for individuals with condition X versus condition Y. Do we know enough about the genomes of our non-model systems to understand how to interpret these data? Will epigenetic factors, for example, maternal antibody transfer, confound our ability to identify genetic associations in wild populations? How useful are Venn diagrams of gene-expression patterns when our real questions involve actually identifying (1) how genotype relates to phenotype and (2) in what way understanding this relationship can allow us to determine the underlying drivers and constraints of evolution? Combining appropriate study systems, time-shift sampling, or following of co-evolutionary cycles, and better understanding of the underlying molecular mechanisms, has the potential to address these questions with respect to the role of host–parasite co-adaptive cycling in maintaining sexually selected traits. Although much of this work could potentially be done using traditional genetics, the fact is that it has not. Many behavioral ecologists have not had the wherewithal to construct the pedigree-based high-density linkage maps of host and parasite required to identify loci responsible for resistance and virulence. High-throughput sequencing technologies combined with phenotypic data can provide this information, thereby allowing hypothesis-driven tests of the limit cycles described by Hamilton and Zuk (1982) to be finally undertaken.

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