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Approaching the Genomics of Risk-Taking Behavior

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Abstract

Individual animals differ in their propensity to engage in dangerous situations, or in their risk-taking behavior. There is a heritable basis to some of this variation, but the environment plays an important role in shaping individuals' risk-taking propensity as well. This chapter describes some of the challenges in studying the genetic basis of individual differences in risk-taking behavior, arguing new insights will emerge from studies which take a whole-genome approach and which simultaneously consider both genetic and environmental influences on the behavior. The availability of genomic tools for three-spined stickleback, a small fish renowned for its variable behavior, opens up new possibilities for studying the genetic basis of natural, adaptive variation in risk-taking behavior. After introducing the general biology of sticklebacks, the chapter summarizes the existing literature on the genetic and environmental influences on risk-taking behavior, and describes the overall strategy that our group is taking to identify inherited and environmentally responsive genes related to risk-taking behavior in this species. Insights gleaned from such studies will be relevant to our understanding of similar behaviors in other organisms, including ourselves.

I. INTRODUCTION

In a wide variety of organisms, individuals differ in their propensity to take risks in different situations. For example, mice show interindividual differences in behavior in an open field (Defries *et al.*, 1966), monkeys vary in aggressiveness (Suomi, 1987), and individual fish differ in their reaction to a predator (Huntingford, 1976). For the purposes of this review, I adopt a very inclusive definition of risk-taking behavior that can encompass this diversity. What is common to all of the examples above is that there is an element of danger involved in the behavior—from the chance of encountering a threat in a new environment, to the probability of injury in fights, to the possibility of death during interactions with a predator. Broadly construed, risk-taking behaviors are expressed in dangerous situations and increase the chance that an individual is injured or even dies.

Individual differences in the propensity to engage in risk-taking behavior have consequences for social groups for several reasons. First, risk-taking behaviors can influence other members of the social group. For example, high levels of aggression can influence other members of a social group by excluding nonaggressive individuals from access to resources, or via the effects of winning or losing fights on subsequent levels of aggression of others (the winner–loser effect). Diverse behaviors such as predator inspection behavior in fishes, mobbing behavior in birds, or alarm calling in rodents could reflect individual differences in the propensity to engage in dangerous situations around predators, and have consequences for the safety of other members of the group. Second, it is likely that the fitness of any given behavioral type (e.g., risk averse versus risk prone) depends on the frequency of other behavioral types within the social group, that is, fitness is frequency dependent. Therefore, understanding the causes and consequences of individual differences in risk-taking behavior would need to take into consideration the social context in which risk-taking behavior is expressed. Third, social dynamics could be influenced by the frequency of risk-taking

phenotypes within the social group. Imagine a group composed entirely of risk-taking behavioral types, versus a group composed of risk-averse behavioral types. It is likely that such groups have different population dynamics and even fitness. Indeed, an intriguing question is whether there might be an optimal combination of behavioral types within the social group (Sih and Watters, 2005). Finally, individual differences in exploratory behavior, or response to novelty, have consequences for dispersal and the colonization of new environments by a founding social group of individuals (Duckworth and Badyaev, 2007).

Individual differences in the propensity to engage in dangerous situations are often influenced by both genetic and environmental factors, and have relevance for human health and disease. Indeed, problems such as self-harm, addiction, sexual risk-taking, and violence have serious adverse consequences in humans. Despite the obvious costs to individuals and society and importance for health, we know relatively little about the etiology of risk-taking behaviors associated with these afflictions. While there are several candidate genes for related behaviors, candidate genes explain only a small fraction of the total genetic variation, suggesting that a whole-genome approach is likely to identify novel genes and pathways that are currently unknown. After describing some of the challenges that confront us as we try to understand the genetic basis of risk-taking behaviors in humans and other organisms, I describe the approach that we are taking to identify inherited and environmentally responsive genes related to risk-taking behaviors in a fresh new model system, three-spined stickleback fish.

II. CHALLENGES AND APPROACHES FOR STUDYING THE GENETICS OF RISK-TAKING BEHAVIOR IN HUMANS AND OTHER ANIMALS

There is a large literature investigating the genetic and neurochemical causes of variation in risk-taking behavior in humans and nonhuman animals. Some of the most recent findings have confirmed that human risk-taking behaviors related to psychopathology are influenced by inherited genetic factors (Kendler *et al.*, 2003). Animal models for related behaviors such as *fearlessness* and *impulsiveness* (Strandberg *et al.*, 2005), *fearfulness* (Boissy, 1995), *aggression* (Miczek *et al.*, 2001), *anxiety and depression* (Adamec *et al.*, 2006; Flint *et al.*, 1995), and *substance dependence* (Crabbe, 2002) have extended these findings by elucidating the mechanisms underlying the specific behaviors. Progress in this field has been facilitated by the use of powerful tools such as microarrays (Kroes *et al.*, 2006) and knockouts (Adamec *et al.*, 2006) to find genes related to behavior.

However, many challenges remain as we try to study the genetic basis of risk-taking behavior, especially in identifying particular genes that might be related to variation in risk-taking behaviors. An immediate challenge is that like all complex traits, it is likely that a large number of genes, each of small effect, are involved in regulating risk-taking behavior (Kendler and Greenspan, 2006; Mackay, 2009). The sample sizes needed to identify quantitative trait loci (QTL) of small effect are prohibitively large ($n > 1000$), especially when we wish to understand how genes are responsive to the environment (Mackay, 2004; Plomin, 2005), and require huge numbers of markers (> 1000) in order to find QTL, making this an approach available to traditional model organisms, such as mouse (reviewed in Hovatta and Barlow, 2008; Willis-Owen and Flint, 2007) and some domesticated animals, for example, chickens (Wiren *et al.*, 2009), quail (Beaumont *et al.*, 2005), and cattle (Gutierrez-Gil *et al.*, 2008).

Indeed, even when candidate genes have been proposed based on neurochemical hypotheses or QTL studies, specific candidate genes (e.g., DRD4, SERT, MAOA) for related behaviors only explain a small fraction of the total genetic variation (Reif and Lesch, 2003), indicating that we have yet to learn the identity of most of the important genes. Another recurring

problem that has plagued candidate gene studies is the failure to replicate. While some studies, for example, find an association between the serotonin transporter polymorphism and behavior, other studies do not (reviewed in Reif and Lesch, 2003). At this point it is unclear how much of the inconsistency among studies is due to methodological problems, that is, population stratification, or due to real genetic differences between populations or species in the genetic mechanisms underlying similar behaviors.

Another challenge is that although there often appears to be some genetic component to risk-taking behavior, it cannot be denied that early experience affects related behaviors in both humans (Farrington, 2005) and nonhuman animals (Caldji *et al.*, 2000; Meaney, 2001), and there is mounting evidence that the environment can influence behavior in a genotype-specific way (G×E interaction) (Caspi and Moffitt, 2006; Eaves *et al.*, 2003). Arguably, the ubiquity (and effect size, >75% of the phenotypic variation in some cases) of genotype by environment interactions (Caspi and Moffitt, 2006; Kaufman *et al.*, 2006) is an indication that studies will have the biggest impact if they simultaneously consider both genetic and environmental factors.

There has been considerable interest in recent years for using gene expression microarrays to identify genes related to behavior. Some of the advantages of measuring whole genome expression using microarrays are that the approach is unbiased and open-ended, and that it considers the coordinated action of the entire genome rather than focusing on one gene at a time. Moreover, microarrays are efficient, in that each microarray is its own self-contained experiment, which facilitates our ability to compare relative expression across genes. Also, microarrays are good for nonmodel systems in which it is not feasible to use traditional forward genetic approaches.

Behavioral experiments using microarrays have compared gene expression following different kinds of experiences, across different behavioral types, or among individuals from different populations or strains. Some studies have compared whole genome expression between groups that have or have not experienced some treatment or challenge that elicits a behavioral response, assuming that whatever genes are differentially expressed are related to the differing behavioral reactions. For example, studies have examined the genomic response to song in birds (London *et al.*, 2009), female response to courtship or mating in *Drosophila* (Carney, 2007; Kapelnikov *et al.*, 2008; Lawniczak and Begun, 2004; Mack *et al.*, 2006; McGraw *et al.*, 2008), swordtail fish (Cummings *et al.*, 2008), and honeybees (Kocher *et al.*, 2008), and male aggression in *Drosophila* (Wang *et al.*, 2008a,b). Related to risk-taking behavior, a number of studies have taken this strategy to identify gene expression correlates of anxiety in mice (Joo *et al.*, 2009; Sherrin *et al.*, 2009; Wang *et al.*, 2008a, b), *Drosophila* (Ibi *et al.*, 2008), monkeys (Sabatini *et al.*, 2007), and rats (Kabbaj *et al.*, 2004; Kroes *et al.*, 2006). However, many questions remain about how to interpret these experiments, how to compare across studies, and what treatment differences imply about the genetics of behavior (see Gibson, 2008). For example, it is difficult to know whether the expression differences are due to the application of the treatment rather than to the execution of the behavior itself. Moreover, the timing of sampling is critical; the genes involved in the immediate genomic response to the treatment could be different from those involved in the execution of the actual behavior. It is also unclear whether the same genetic mechanisms underlie treatment level differences and behavioral differences between individuals.

Another approach is to compare baseline gene expression differences between different genotypes or individuals that differ in behavior, assuming that whatever expression differences observed are related to behavioral differences (Aubin-Horth *et al.*, 2005; Ben-Shahar *et al.*, 2002; Dierick and Greenspan, 2006; Edwards *et al.*, 2006; Gammie *et al.*, 2007; Kim *et al.*, 2007, 2009; Quilter *et al.*, 2008; Renn *et al.*, 2008; Wang *et al.*, 2008a, b;

Whitfield *et al.*, 2003). However, differences in transcript abundance could be a consequence, rather than a cause of differences in behavior. Despite this caveat, showing differential expression can be an important first step toward identifying the causative genetic polymorphisms underlying the difference in gene expression.

III. APPROACHING THE GENOMICS OF RISK-TAKING BEHAVIORS IN STICKLEBACK FISH

Obviously, we still have a lot to learn about the etiology of risk-taking behaviors in humans and other organisms. While traditional animal models have been useful in identifying mechanisms underlying some behaviors, such studies have typically been carried out on genetically homogeneous and lab-adapted strains. New insights might emerge from studying natural variation in behavior that resembles human behavioral variation.

Three-spined sticklebacks (*Gasterosteus aculeatus*) are renowned for their natural variation in behavior, morphology, and physiology (Bell and Foster, 1994). Therefore, in our work, we are using three-spined sticklebacks to identify candidate genes for natural variation in risk-taking behaviors. Sticklebacks are small (4–5 cm standard length at maturity) teleost fish that occur in the northern hemisphere. Sticklebacks commence breeding in the spring and typically live for 1 year. We are taking a whole-genome approach to understand genetic and environmental effects on suites of covarying risk-taking behaviors.

Our hypothesis is that there are inherited and environmentally responsive genes that affect risk-taking behaviors in sticklebacks, and those genes are shared with other animals, including humans. Later, we describe how risk-taking behaviors in this species show adaptive, natural variation within and between populations. Behaviors such as aggression and predator inspection are influenced by both inherited and experiential factors (Tulley and Huntingford, 1987), and they covary (Bell, 2005; Bell and Stamps, 2004; Huntingford, 1976). Moreover, the species is experimentally tractable—a favorite subject of the classical ethologists (Tinbergen, 1972; Wootton, 1984), the behaviors of sticklebacks are well characterized and are amenable to laboratory investigation. In addition, there are genomic resources for sticklebacks, including a whole-genome sequence (11× coverage by the Broad Institute), BAC and cDNA libraries, an EST project and a whole genome-linkage map (Peichel *et al.*, 2001).

Sticklebacks have another key attribute that makes them an especially good model: they have an unusual evolutionary history that has produced a replicated natural experiment. Freshwater populations of sticklebacks are the descendants of marine ancestors which independently colonized freshwater environments following glacial retreat ~12,000 years ago. Isolated postglacial freshwater populations rapidly adapted to local environmental conditions, resulting in incredible phenotypic diversity among close relatives (Bell and Foster, 1994). Independent populations rapidly evolved convergent phenotypes in response to similar selective pressures, such that the same traits arose repeatedly and independently from the same ancestor. This system has already had great success in identifying the genetic basis of morphological traits (Colosimo *et al.*, 2005; Cresko *et al.*, 2004; Peichel *et al.*, 2001; Shapiro *et al.*, 2004).

This important feature of the stickleback system means that we have a built-in solution to a problem that has plagued candidate gene studies: the failure to replicate. After we identify a set of genes associated with risk-taking behaviors, we can replicate the experiment by comparing the genes in additional sets of populations. If the same genes are differentially expressed between an independent pair of risk-averse and risk-prone populations, we can

conclude that the differential expression is related to the behavior and is not simply a consequence of other factors such as genetic drift (Fig. 4.1).

A. Risk-taking behavior in sticklebacks

In the following sections, I describe how sticklebacks show natural variation in risk-taking behaviors such as predator inspection and aggression which are amenable to manipulative experimentation, and which resemble familiar human tendencies such as sensation-seeking, fearlessness, and disinhibition. Like the spectrum of externalizing behaviors (conduct disorder, antisocial personality, and substance abuse) in humans (Krueger *et al.*, 2002), risk-taking behaviors in sticklebacks also covary and are influenced by both heredity and early life stress. Another key similarity between the stickleback model of risk-taking and human externalizing behaviors is that they share the same neuroendocrine substrates, as described below. The recent availability of new genomic tools for sticklebacks means that these ecologically relevant behaviors are genetically tractable.

1. Individuals differ in their propensity to take risks in different situations—A consistent result of all of our studies is that there is substantial variation among both wild-caught and lab-reared individuals in how they behave in dangerous situations. For example, in an assay similar to the open field test (Yalcin *et al.*, 2004), some individual sticklebacks actively move around an unfamiliar and dangerous environment while others scarcely leave the safety of a refuge (Fig. 4.2). Therefore, we can use genetic correlates of this individual variation as a tool for finding candidate genes related to risk-taking behavior.

Another dangerous situation in which we observe individual differences in behavior is during confrontation by a potential predator. While some individuals hide in the presence of a predator, others swim up to the predator's mouth and face the predator head-on. The same individuals that engage in this dangerous behavior, known as predator inspection, are also relatively aggressive toward other sticklebacks and are more willing to take risks in order to gain food than their risk-averse conspecifics (Bell, 2005; Bell and Stamps, 2004; Huntingford, 1976).

Individual sticklebacks can be classified as either risk-prone or risk-averse in several other contexts. Predator inspection is one of the most obvious forms of risk-taking behaviors and has been widely studied in sticklebacks and other small fishes (e.g., Pitcher *et al.*, 1986). Despite the obvious danger involved in performing this behavior (Dugatkin and Godin, 1992; Milinski *et al.*, 1997), it is thought that predator inspection can provide reliable information about predation risk via both olfactory and visual cues.

Individual differences in risk-taking behavior are also observed when individuals balance the benefits of feeding against the costs of potential predation (Bell, 2005). Some risk-prone individuals are more willing to assume the risk of predation in order to get food than others, even when differences in size, sex, or hunger level are controlled for (e.g., Krause *et al.*, 1998). Therefore, differences in the willingness to forage in the presence of a predator can reflect intrinsic differences in the propensity to engage in risk-taking behaviors.

Fighting with conspecifics is another dangerous situation in which individual differences in risk-taking behavior are observed (Bell, 2005; Bell and Stamps, 2004; Huntingford, 1976). Intraspecific aggression in sticklebacks occurs during competition over access to resources, including food and territories and is manifested as biting, chasing, and attacking conspecifics. These behaviors are dangerous because in addition to energetic costs (Thorpe *et al.*, 1995), aggression can result in injury (Neat *et al.*, 1998) and exposure to predators while fighting (Diaz-Uriarte, 1999). The territorial aggression of male sticklebacks is

especially well characterized (Bakker, 1994), but juveniles and females can also be aggressive (Bakker, 1986).

Interestingly, in sticklebacks, individuals that engage in higher levels of predator inspection are also more aggressive toward conspecifics (Bell, 2005; Bell and Stamps, 2004; Huntingford, 1976). Such covariation has parallels with the externalizing spectrum in humans, and with the observation that diverse psychiatric disorders in humans co-occur; antisocial behaviors, substance dependence, impulsivity, and behavioral disinhibition are comorbid (Kendler and Greenspan, 2006). Covariation among behavioral responses to dangerous situations in sticklebacks suggests that there is variation in the tendency to engage in risk-taking behaviors, and this tendency is manifested in multiple contexts (Sih *et al.*, 2004).

In humans, there is evidence that the broad, underlying tendency is more heritable than the particular manifestations (Krueger *et al.*, 2002). For example, whereas a significant portion of the variance in the externalizing latent trait can be traced to common genetic factors (>80% of the variance; Krueger *et al.*, 2002), heritabilities for single behaviors (e.g., conduct disorder, alcohol dependence) are generally much lower. If the same is true for risk-taking behaviors in sticklebacks, we might be more likely to find genes related to risk-taking behaviors if we look for genetic correlates of the propensity to be generally risk-averse or risk-prone.

2. There is a genetic basis to risk-taking behaviors in sticklebacks—Several lines of evidence suggest that there is a genetic component to risk-taking behaviors in sticklebacks. First, risk-taking behavior is repeatable. Figure 4.3 shows the results of an experiment in which different individual sticklebacks were measured for their behavioral reaction to a live fish predator (pike) on several occasions (Bell *et al.*, 2009). Individuals differed in the total amount of time they spent freezing (remaining motionless) in the presence of a predator. Freezing is negatively related to risk-taking behaviors and we interpret it as reflecting fearfulness. Individual differences in freezing were consistent through time. Specifically, individuals that froze in response to the predator the first time they were measured also froze when measured on subsequent occasions. These data are important because they show that an individual's willingness to take risks is a stable attribute, supporting the hypothesis that there is a genetic basis to risk-taking behaviors in sticklebacks (Boake, 1994).

Another line of evidence for a genetic component to risk-taking behavior is that there is significant genetic variation for risk-taking behavior among families (Bell, 2005; Bell and Stamps, 2004). When confronted with an unfamiliar environment, members of certain families actively explored the environment while members of other families did not. As can be seen from the data in Fig. 4.4, there is substantial variation among families within populations in their willingness to explore an unfamiliar environment. These data are important because they show that there is heritable genetic variation within stickleback populations for risk-taking behaviors.

Common garden experiments also show that there is a genetic basis to risk-taking behaviors (Bell, 2005; Bell and Stamps, 2004). As can be seen from the data in Fig. 4.5, sticklebacks from one population were more willing to eat in the presence of a predator compared to sticklebacks from another population. This difference was apparent both for wild-caught parents and their lab-reared offspring that were reared under standardized laboratory conditions. These data are important because they show that the behavioral difference between the two populations has a genetic component. Moreover, it illustrates another important point about sticklebacks: the two populations differ in predation pressure which is

a major source of variation for many stickleback phenotypes, and is discussed further later (Huntingford *et al.*, 1994; Reimchen, 1994).

A heritable basis to behaviors that deter predation (antipredator behaviors) is further substantiated by other studies on fishes, which have shown that differences between populations in antipredator behavior are inherited and arise without any experience of predators or without exposure to threatening situations (Magurran, 1990). There is also a heritable basis to aggression in sticklebacks (Bakker, 1986).

3. Risk-taking behavior is also influenced by the environment—However, we also know that risk-taking behaviors in sticklebacks are influenced by the environment, including early interactions with parents. During the reproductive season, which is triggered by lengthening day lengths and increasing temperatures, male sticklebacks defend nesting territories. Males attract females to spawn in their nests and defend the breeding territory from intruders and predators. After spawning, the female leaves the male's territory and the male is solely responsible for the care of the eggs. During the ~9-day incubation period, the male “fans” (oxygenates) the eggs, removes rotten eggs and debris, and defends the territory. A breeding male stickleback tends his newly hatched offspring for ~10 days. The fathers chase and catch fry that stray from the nest and spit them back into the nest. The fry are not injured during these interactions, but Tulley and Huntingford (1987), following a suggestion by Benzie (1965), showed that these interactions help to prepare the stickleback fry for later encounters with predators. Figure 4.6, reproduced from Tulley and Huntingford (1987), shows that sticklebacks reared by their father (“N”) avoided a model pike predator more than sticklebacks reared without paternal care (orphans, “O”). Interestingly, the behavioral difference between father-reared and orphan sticklebacks was only apparent among sticklebacks from a population where predators are abundant (the Mar Burn population on the left).

In addition to the influence of their father, young fishes also learn from their own direct experience with predators, and these early interactions affect subsequent risk-taking behaviors later in life (e.g., Vilhunen, 2006). Interestingly, the effect of both paternal care and direct experience appears to be especially strong for fish from high predation localities. The fact that fish from high predation localities learn faster about predators suggests an inherited predisposition to respond to experience (Huntingford and Wright, 1992). Similar G×E interactions, in which some genotypes (or populations) are more responsive to the environment, have been found for risk-taking behaviors in other fish species (e.g., Gerlai and Csanyi, 1990).

4. Sticklebacks from populations experiencing higher levels of predation engage in more risk-taking behaviors than their counterparts from safer environments—As mentioned above, sticklebacks are widely distributed throughout the northern hemisphere and have a penchant for rapidly adapting to their local environments. Populations that occur in similar environments have evolved similar behaviors. One of the most important selective forces shaping stickleback populations is predation pressure. That is, some lakes contain many predators which prey on sticklebacks (“high predation”) while other lakes are relatively predator-free (“low predation”). We have been comparing the risk-taking behaviors of sticklebacks in a set of populations in Scotland that vary in predation pressure (Bell *et al.*, 2009). Scotland is especially well suited for studying variation in risk-taking behavior in response to predation pressure because the country is teeming with postglacial waterbodies. Many lochs and ponds have been isolated for long enough (up to 15,000 generations) to independently evolve adaptations to high or low predation pressure and to become genetically differentiated from each other, but still capable of interbreeding (Malhi *et al.*, 2006).

Interestingly, sticklebacks from areas where there are high levels of predation tend to be more risk-prone (i.e., they show higher levels of risk-taking behaviors) than their counterparts in safer environments (Huntingford and Coulter, 1989; Huntingford *et al.*, 1994; Walling *et al.*, 2003, 2004). This pattern has been documented in other small fish species as well, such as guppies (Magurran, 1986). While risky behavior in a dangerous environment might seem nonintuitive, this result is predicted by life history theory. The reason is that small individuals are especially vulnerable to predation, so when predation pressure is high, individuals that grow quickly will be favored because they are not small and vulnerable for long. Therefore, risk-taking behaviors that improve growth rate such as active foraging and aggression that results in access to resources should be favored when predation pressure is high (Mangel and Stamps, 2001). It is also worth noting that increased levels of risk-taking behaviors in humans have been documented in harsh or impoverished environments (Farrington, 2005; Kendler *et al.*, 1995).

5. Risk-taking behaviors in sticklebacks resemble risk-taking behaviors in other organisms—Our contention that the genetic mechanisms underlying risk-taking behaviors in sticklebacks are shared with humans and other animals is supported by evidence that the neuroendocrine mechanisms underlying risk-taking behaviors are the same. If we can show that the neuroendocrine substrate underlying risk-taking behaviors in sticklebacks are conserved, then it is likely that the candidate genes that are identified in the stickleback system are likely to be good candidates for other animals, including humans.

For example, risk-taking behavior in sticklebacks is associated with serotonin turnover (Bell *et al.*, 2007). As can be seen in Fig. 4.7, individuals that engaged in more frequent predator inspection behavior had lower serotonergic activity. Low serotonergic activity is associated with sensation seeking in humans (Netter *et al.*, 1996) and aggression in several other animals (Higley *et al.*, 1996).

Second, breathing rate (opercular beats) is predictive of an individual's risk-taking tendency in sticklebacks. We measured opercular beats in response to handling stress and then the same individuals' behavioral reaction to a predator 24 h later. As can be seen from the data in Fig. 4.8, fish that breathed faster in response to handling stress were more likely to engage in risk-taking behavior (Bell *et al.*, 2009). Unlike some other physiological measures, opercular beats can be measured noninvasively and repeatedly on the same individuals. Therefore, opercular beat rate is an appealing possible endophenotype linking genes and behavior. Studies on other organisms have also found associations between metabolic rate and risk-taking behaviors (Carere and Van Oers, 2004; Heim and Nemeroff, 2001).

6. Genomic resources for sticklebacks—Genomic resources for stickleback research are rapidly expanding. The first release of the stickleback genome, sequenced and assembled at the Broad Institute, comprises 460 Mb. Gene models include approximately 21,000 known, novel and pseudogenes with an estimated 44,000 Genscan predictions. Furthermore, close to 250,000, 3' and 5' expressed sequence tags (ESTs) from various tissues and developmental stages have been submitted to GenBank and comprise 15,087 Unigene clusters (Kingsley *et al.*, 2004).

IV. FUTURE DIRECTIONS AND CONCLUSIONS

Based on the work described earlier, we are taking a multipronged strategy for investigating the genetic correlates and causes of risk-taking behaviors in sticklebacks. We hypothesize that inherited and environmentally responsive genes that affect risk-taking behaviors in sticklebacks have orthologs with common function in other animal genomes, including human. Therefore, using whole genome microarrays, we are searching for candidate genes

related to the propensity to engage in risk-taking behaviors that are both inherited and responsive to the environment. First, we are comparing baseline brain gene expression differences between risk-prone and risk-averse individuals and populations. Second, we are also identifying genes underlying risk-taking behaviors that are responsive to adverse environmental conditions by comparing the brain gene expression profiles of individuals exposed to different early life stressors. Third, we are testing candidate genes related to risk-taking behaviors in replicated populations of sticklebacks.

Future studies on the genetics of risk-taking behaviors in sticklebacks and other animals should focus on understanding the mechanisms by which candidate genes influence the behavior. Manipulations such as morpholinos, RNAi, and transgenics (Hosemann *et al.*, 2004; Kingsley *et al.*, 2004) will help to make the causal link between candidate genes and risk-taking behaviors; ultimately, making a risk-averse individual risk-prone. The future is also bright for whole genome association studies to study the genetic basis underlying phenotypes in natural populations, such as being done already in humans (Butcher *et al.*, 2008; Dina *et al.*, 2005; Donner *et al.*, 2008; Doyle *et al.*, 2008; Stallings *et al.*, 2005) and dogs (Jones *et al.*, 2008). In the not-too-distant future, it is likely that the main barrier to progress will not be genotyping our subjects, but in phenotyping the thousands of animals needed in order to obtain sufficient power to detect relationships between minor loci and behavior.

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Figure 4.1.

The replicated evolution of risk-taking behaviors in sticklebacks provides a natural experiment for testing candidate genes. This idealized map of Scotland shows the movement of sticklebacks from the ocean (on left) into freshwater rivers following glacial retreat. Sticklebacks inhabiting water bodies with abundant predators independently evolved increased levels of risk-taking behavior (dark fish) compared to sticklebacks inhabiting water bodies without predators (light fish).

Figure 4.2.

Individuals vary in their propensity to take risks. This histogram shows the distribution of exploratory movements of individuals in the presence of a predator. Methods: Juvenile sticklebacks from several populations were brought into the lab and the number of times an individual moved during 15 min in the presence of a predator was recorded. Mean=4.6, S.D.=3.39, $n = 218$.

Figure 4.3.

Risk-taking behavior in sticklebacks is repeatable. The Y-axis shows the rank order time spent freezing in the presence of a live predator. Time (the observation number) is on the X-axis. Each line represents a different individual; each point represents the measure of that individual on each observation day. Methods: On seven different occasions over the course of 2 months, individual sticklebacks were presented with a live pike and their behavioral response recorded. Repeatability was calculated as in Lessels and Boag (1987), $R=0.68$, $F_{6,42}=13$, $p<0.001$. From Bell *et al.* (2009).

Figure 4.4.

There is genetic variation for risk-taking behaviors among families. The data show the mean ($\pm$ S.E.) number of exploratory movements in an unfamiliar environment of different families from two different populations, Putah Creek (gray) and the Navarro River (black). Methods: The exploratory behavior of lab-reared sticklebacks from 22 different families (2–5 full sibs/family) were measured at 7 months of age. Data were analyzed with a nested ANOVA with family and population as fixed factors (Population: $F_{21,102} = 21$, $p < 0.0001$; Family(Population): $F_{20,102} = 2.1$, $p = 0.003$).

Figure 4.5.

There is a genetic basis to behavioral differences between populations. These data show the standardized mean ($\pm$ S.E.) number of bites at food while in the presence of a predator for wild-caught parents and their lab-reared offspring from two different populations, Navarro and Putah (Bell, 2005). Methods: The willingness to forage in the presence of a predator was measured on wild-caught adults from the two populations. Full-sib offspring from the two populations were reared under standardized laboratory conditions and measured for willingness to forage in the presence of a predator at adulthood. Both the parents and offspring of fish from Putah Creek took more bites in the presence of a predator (Parents: $F_{1,75}=14.3$, $p < 0.0001$; Offspring: $F_{1,44}=18.4$, $p < 0.0001$).

Figure 4.6.

Paternal care improves antipredator behavior. This is Fig. 1 from Tulley and Huntingford (1987). The strength of avoidance shown to a model pike by sticklebacks from the Mar Burn, where predators are abundant, and from Inverleith Pond, where predators are absent with ("N") and without ("O") paternal care.

Figure 4.7.

Risk-takers have lower serotonergic activity. Predator inspection was negatively correlated with serotonergic activity, as measured by the turnover of serotonin (5-HT) to its metabolite 5-hydroxyphenylacetic acid (5-HIAA), 15 min after presentation of the predator. From Bell *et al.* (2007). Methods: Juvenile sticklebacks were measured for their behavioral reaction to a live pike predator and then sacrificed after 15 min, their brains removed and quickly deep frozen. Tissue was homogenized in 4% perchloric acid with an internal standard and monoamines were measured using HPLC with electrochemical detection as in Øverli *et al.* (1999). The correlation between individual levels of predator inspection and serotonergic activity was statistically significant ($r = -0.669$, $n = 9$, $p = 0.049$).

Figure 4.8.

Breathing rate is an endophenotype for risk-taking behavior. The number of opercular beats in 15 s after handling is positively correlated with predator inspection (Bell *et al.*, 2009).

Methods: Individuals were subjected to brief handling stress and the number of opercular beats was counted. At least 24 h after, the fish was presented with a live pike and the number of predator inspections in 15 min was recorded. The partial correlation coefficient (controlling for population) is statistically significant ($r=0.211$, $n=168$, $p=0.006$) and was not related to body size.