



K O N I N K L I J K E N E D E R L A N D S E
A K A D E M I E V A N W E T E N S C H A P P E N

Genomic tools for behavioral ecologists to understand repeatable individual differences in behavior

Bengston, Sarah; Dahan, Romain; Donaldson, Zoe; Phelps, Steven; van Oers, K.; Sih, Andrew; Bell, Alison

published in

Nature Ecology and Evolution
2018

DOI (link to publisher)

[10.1038/s41559-017-0411-4](https://doi.org/10.1038/s41559-017-0411-4)

document version

Peer reviewed version

[Link to publication in KNAW Research Portal](#)

citation for published version (APA)

Bengston, S., Dahan, R., Donaldson, Z., Phelps, S., van Oers, K., Sih, A., & Bell, A. (2018). Genomic tools for behavioral ecologists to understand repeatable individual differences in behavior. *Nature Ecology and Evolution*, 2, 944-955. <https://doi.org/10.1038/s41559-017-0411-4>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

pure@knaw.nl

1 Genomic tools for behavioral ecologists: advancing the understanding of repeatable
2 individual differences in behavior

3

4 Sarah Bengston^{1,2}, Romain Dahan^{2,3}, Zoe Donaldson⁴, Steve Phelps⁵, Kees van Oers⁶,
5 Andrew Sih⁷, Alison Bell⁸

6

7 1: Department of BioSciences, Rice University, Houston, TX USA 77005

8 2: Department of Biology, University of Rochester, Rochester, NY USA 14611

9 3: School of Life Sciences, Arizona State University, Tempe, AZ USA, 85281

10 4: Department of Department of Molecular, Cellular, and Developmental Biology, and
11 Department of Psychology and Neuroscience, University of Colorado Boulder, Boulder
12 CO USA, 80309

13 5: Department of Integrative Biology, University of Texas Austin, Austin, TX USA
14 78712

15 6: Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
16 Wageningen, The Netherlands

17 7: Department of Environmental Science and Policy, University of California Davis,
18 Davis, CA USA 95616

19 8: Department of Animal Biology, The University of Illinois, Urbana-Champaign,
20 Urbana, IL USA 61801

21

22

23 **Summary**

24 Behavior is a key interface between an animal's genome and its environment. Repeatable
25 individual differences in behavior have been extensively documented in animals, but the
26 molecular underpinnings of behavioral variation among individuals within natural
27 populations remain largely unknown. Here, we offer a critical review of when molecular
28 techniques may yield new insights, and we provide specific guidance on how and
29 whether the latest tools available are appropriate given different resources, system and
30 organismal constraints, and experimental designs. Integrating molecular genetic

31 techniques with other strategies to study the proximal causes of behavior provides
32 opportunities to expand rapidly into new avenues of exploration. Such endeavors will
33 enable us to better understand how repeatable individual differences in behavior have
34 evolved, how they are expressed, and how they can be maintained within natural
35 populations of animals.

36

37

38 **The molecular genetic basis for repeatable behavioral variation**

39 The stunning diversity of behavior *within* a species has become a thriving area of
40 research for behavioral ecologists. As a result, we now know that repeatable **individual**
41 **differences in behavior** among animals within populations is ubiquitous. Studies under
42 the umbrella of animal personality (reliable differences in behaviors across contexts or
43 time) and behavioral syndromes (correlated behavioral traits)¹⁻⁴ have yielded thousands
44 of publications, particularly during the last decade, and lay a solid foundation for
45 understanding the evolution and effect of repeatable variations in behavior both
46 theoretically and empirically. Nevertheless, fundamental questions remain unresolved.
47 (1) Why are individuals consistent at all? In other words, why is behavior not infinitely
48 plastic? (2) Why are some behaviors correlated? And why do correlations sometimes
49 vary among individuals and populations? (3) What explains individual differences in
50 developmental plasticity (effects of earlier experiences on subsequent behavioral
51 tendencies)? Differences in contextual plasticity (effects of current conditions on
52 behavior)? (4) And finally, why do individuals have different behavioral types? Indeed,
53 knowing how and whether selection acts on consistent among-individual differences in
54 behavioral traits has important implications for our understanding of the maintenance of
55 variation within natural populations, a central problem in evolutionary biology.

56 Genomics and other molecular methods have many applications for studying the
57 mechanistic basis of animal behavior, which have been outlined elsewhere (e.g. Rittschof
58 and Robinson 2014⁵). This article focuses on the application of such tools to repeatable
59 individual variation in behavior. Fundamental questions about behavioral variation can be
60 difficult to resolve without some understanding of genetic or physiological variation,
61 often upstream to behavior. While downstream processes may also affect behavioral
62 variation (e.g. differences in the peripheral nervous system affect task allocation in
63 *Temnothorax* ant workers⁶), if looking at the appropriate time point, molecular organizing
64 signatures may still be detected, either if they are the result of environmental signaling or
65 individual genetic differences. As interest in integrating studies of proximate and ultimate
66 mechanisms increases, researchers interested in behavioral variation have begun applying
67 these methodological approaches across Tinbergian levels of analysis⁷⁻⁹.

68 Genomics has revolutionized our understanding of evolution, ecology and
69 physiology, yet even with recent advances, the study of animal behavior has been slower
70 to embrace genomic technologies. One possible reason is that until recently the relevant
71 genetic tools have been out of reach for animal behaviorists fascinated by the behavioral
72 diversity within and among non-model species (Box 1). In addition, repeatable behavioral
73 variation is likely the result of multifaceted, highly dynamic, and non-linear epistatic,
74 transcriptional, epigenomic, ontogenetic, neural, and metabolic processes¹⁰, which makes
75 it hard to study. Plastic traits such as behavior present specific challenges for studies at
76 the molecular level: compared to morphological and most life history traits, behavior is
77 repeatedly expressed, meaning there can be significant trait plasticity within an
78 individual. Plasticity itself can also vary between individuals^{11,12}. The phenotypic
79 gambit¹³ and a relative lack of integration across Tinbergen's levels of analysis^{14,15} has
80 also slowed progress in this area. Moreover, there is skepticism in some circles about
81 whether we need to study traits (including behavioral traits) at the molecular level at all¹⁶⁻
82 ¹⁸, and if the benefits outweigh the considerable costs, both in terms of monetary expense
83 and the training required for proficiency^{16,18}. Indeed, some fundamental questions about
84 repeatable individual variation in behavior do not require expensive forays into the world
85 of genomics. For example, if the researcher is interested in the mechanisms underlying a
86 behavior, there may be few compelling reasons to incorporate genomics if there are
87 already candidate genes related to the behavior of interest¹⁹. In addition, we can learn a
88 lot about behavioral evolution (i.e. does behavioral variation reflect genetic or
89 environmental causes?) using standard quantitative genetic "gene free" approaches (such
90 as cross-fostering or common garden experiments) without incorporating genomics.

91 However, there are compelling reasons for evolutionary biologists to investigate
92 the genetic and molecular mechanisms of behavioral variation. For example, both
93 environmental and genetic variation shape behavior, but whether these effects share
94 overlapping molecular mechanisms remains unclear. As such, identifying the genes that
95 contribute to environmentally and genetically-mediated variation has major implications
96 for understanding the evolution of phenotypic plasticity²⁰. In addition, once we have
97 identified these genes, we can begin to ask fundamental questions about pleiotropy²¹, i.e.
98 whether the same genes influence multiple traits, and whether these genes are under

99 selection²². Finally, as we examine the genetic basis the same “trait” across species, we
100 can begin to discover whether the molecular mechanisms underlying behavioral variation
101 are deeply conserved in evolution²³.

102 There are already hints that applying genomic tools to study behavior at the
103 molecular level can offer fundamental insights. For example, in an early microarray
104 paper, Whitfield and colleagues were able to track the enormous transcriptomic plasticity
105 (39% of the genes expressed in the brain) that contributes to age polyethism in honey bee
106 workers²⁴. Through QTL mapping, Bendesky and colleagues recently identified 12
107 genomic regions associated with variation in parental care in *Peromyscus* mice, some of
108 which had sex-specific effects, which provides a plausible mechanism by which parental
109 behavior can evolve independently in the sexes²⁵. It is only a question of time until these
110 tools are applied to address fundamental questions about behavioral variation *among*
111 individuals within populations. For example, understanding why traits such as aggression
112 and exploratory behavior are often correlated has been hypothesized to either be the
113 result of pleiotropic interactions or linkage with “aggressiveness genes” favored in
114 environments where conspecific aggression is beneficial^{2,7}. The underlying causes of
115 such so-called spillovers have rarely been tested, though with knowledge of underlying
116 mechanisms, it is in principle an empirically tractable question. Similarly, a fundamental
117 question often asked about repeatable behavioral variation – why is an individual’s
118 behavior consistent rather than infinitely plastic? – is often attributed to the costs of
119 plasticity (but see Stamps and Frankenhuis 2016²⁶)²⁷. However, measuring the fitness
120 costs of behavioral plasticity has proven to be difficult²⁸. Genomic technologies might be
121 fruitfully brought to bear on this question by asking about the extent of transcriptomic
122 remodeling in different tissues in response to ecologically-relevant behavioral stimuli,
123 including predators, conspecifics, parasites, or novel environments.

124 **Introduction to the tools**

125 For behavioral ecologists venturing for the first time into the unfamiliar territory
126 of using molecular approaches, the process can be daunting. Selecting the right high-
127 throughput genomic technology for a project requires careful consideration of the
128 questions posed, resources available, and of the limitations presented by a given tool and
129 a given system. Contemporary approaches examine three levels of genomic variation:

130 genetic, epigenetic, and transcriptional. Genetic variation corresponds to sequence
131 differences. Epigenetic variation is evidenced in differences in the molecular marks on
132 DNA and DNA chaperone proteins, which affect DNA accessibility to transcription
133 factors. Transcriptional variation refers to differences in the amount of RNA generated
134 from a particular DNA locus. Variation at any of these levels is likely are not
135 independent from each other. For instance, transcriptional variation is likely tide to
136 genetic or epigenetic variation, making parallel approaches that examine multiple levels
137 of variation a potentially powerful approach.

138 There are three main categories of molecular tools: 1) **genotyping molecular**
139 **markers**, which involves the widespread sequencing of genomic DNA across
140 phenotypes, populations, and/or species; 2) **comparative gene regulation** studies, which
141 characterize transcription and its epigenetic regulation; and 3) **genetic manipulations**,
142 which directly test function. While not specifically a genomic tool, we will additionally
143 discuss **whole genome sequencing**, as the presence of a sequenced, assembled and
144 annotated genome can significantly affect how useful specific tools may be, but is not
145 necessarily a trivial task to accomplish. **Table 1** presents some practical considerations
146 for using these tools. These are, of course, not the only considerations, and further
147 information relevant to tool selection is considered below.

148

149 *Genotyping molecular markers for gene association studies*

150 Contemporary genotyping methods involve the widespread genotyping of markers
151 across the genome, and can be used to directly compare genome structure (e.g.
152 supergenes) and content of different phenotypes at an unprecedented resolution²⁹. These
153 methods involve the targeted enrichment of specific, known sequences (whole exome
154 sequencing, targeted enrichment), or the targeting of randomly distributed restriction sites
155 across the genome (RAD-Seq; Genotyping-by-Sequencing).

156 Genome-wide association studies (GWAS) involve searching the genome for
157 marker polymorphisms that are associated with variation in the phenotype of interest.
158 Once markers have been identified, targeted sequencing and/or comparison to a reference
159 genome might reveal the location and identity of genes that lie within loci that segregate
160 with the trait. An advantage of GWAS is that it can be carried out in wild, unmanipulated

161 natural populations, and does not require performing specific crosses. However,
162 considering the great number of comparisons and correlations tested using markers across
163 the genome (for linkage maps, genome-wide association studies, etc.), the detection of
164 genomic regions in significant associations with trait variation often requires an
165 extremely large sample size (10,000 - 100,000+)²⁹. A large sample size is also important
166 for accurately estimating allele frequency in the population³⁰.

167 Quantitative trait loci (QTL) mapping involves generating crosses between
168 behaviorally divergent individuals and then tracking the segregation of markers linked to
169 phenotypic variation across several generations³¹. The power of QTL mapping is limited
170 by the number of meioses that shuffle associations between markers and the causal
171 alleles, and by the number of loci contributing to variation in the trait and their respective
172 effect sizes; sample sizes on the order of several hundreds to thousands of F2s are
173 required to detect loci of medium effect³². QTL mapping also requires that hybrids are
174 viable, and requires that animals can be reared under laboratory conditions for several
175 generations.

176 Gene association studies via either QTL mapping or GWAS are most likely to be
177 successful when variation is discrete, highly heritable and affected by relatively few loci
178 of large effect. For example, in ruffs there are three alternative male mating morphs under
179 negative frequency-dependent selection. This inversion of a supergene was mapped using
180 only 41 individuals³³. Unfortunately, most repeatable behavioral variation within
181 populations is continuous, rather than discrete, and is likely to be underlain by thousands
182 of genes of small effect. Therefore, crosses between species or populations with discrete
183 variation might be more tractable for mapping. For example, QTL associated with
184 variation in parenting behavior between two sister species of mice was found using this
185 approach²⁵.

186

187 *Comparative gene regulation*

188 Transcriptional profiling (e.g. RNA-Seq) can be used to compare gene expression
189 across phenotypes or conditions in order to identify associations between the expression
190 of specific genes or gene pathways and behavioral phenotypes^{34,35}. Transcriptomics is a
191 particularly appealing genomic tool for plastic behavioral traits because measuring gene

192 expression (possibly in combination with quantifying static DNA sequence variation) can
193 reveal how the genome dynamically responds to the environment, including the social
194 environment ³⁶. For example, studies have compared gene expression between
195 animals that either have or have not experienced a change in their environment that
196 causes changes in behavior. Such studies have shown that the genome is remarkably
197 dynamic: roughly ~10% of the genome responds to a **mating opportunity** ³⁷⁻⁴²,
198 **predation risk** ⁴³⁻⁴⁶, or a **territorial challenge** ⁴⁷⁻⁴⁹.

199 An advantage of transcriptional profiling over GWAS is that lists of differentially
200 expressed genes can be generated with much smaller sample sizes (on the order of a
201 typical behavior experiment), and therefore can be an accessible point of entry for
202 behavioral ecologists into the world of genomics. However, one challenge of using
203 transcriptional profiling is that it results in an unbiased list of often hundreds of
204 differentially expressed genes, requiring thoughtful consideration of how best to move
205 forward with investigation of candidate genes. Lists of differentially expressed genes are
206 a far cry from knowing the specific causal variants underlying phenotypic variation, and
207 results are highly contingent on where tissue is sampled because gene expression is
208 highly cell type-specific. Developmental genes are more likely to be expressed at specific
209 embryonic, larval, or pupal stages (though genes originally described as developmental
210 may also be expressed in adults ^{23,50}), and gene expression involved in behavioral traits
211 may vary within the specific neuronal circuits that subserve that behavior. Given these
212 considerations, it is perhaps not surprising that gene expression is also known to vary
213 between field- and lab-reared organisms⁵¹, which calls for thorough validation of lab-
214 based results in natural populations.

215 The destructive nature of most tissue sampling (especially for brain) poses
216 challenges for obtaining repeated samples, and might not be realistic for long-term
217 studies of marked individuals, or studies with threatened species. An emerging alternative
218 involves using peripheral proxy tissues such as blood^{52,53}. This can be useful, but comes
219 with its own limitations. For example, blood measures of gene expression are generally
220 not relevant to expression within circuits of the nervous system; gene expression varies
221 tremendously even between neuronal cell types and brain regions, so looking at tissue as
222 different as blood is unlikely to reveal transcriptional variation driving behavior in the

223 brain⁵⁴ (though, this may depend on the type of gene⁵⁵). However, blood gene expression
224 is influenced by many of the same factors that regulate brain gene expression, including
225 rearing environment, stress and diet. Viewed as markers for repeatable behavioral
226 variation, rather than as causal contributors to behavioral variation, such studies can be
227 informative.

228 There is also growing appreciation that gene expression patterns are highly
229 contingent on *when* tissue is sampled because gene expression can change quickly – on
230 the scale of minutes to hours- and we know very little about the arc of this time course
231 (see Rittschof et al. in review). Therefore differences between phenotypes or
232 experimental treatments may exhibit distinct patterns at 30 or 120 minutes⁵⁶ after a
233 behavioral interaction, for example. The molecular responses to behaviorally relevant
234 stimuli likely involve waves of transcription associated with various types of behavioral
235 plasticity (detecting the stimulus, assessing the stimulus, responding to the stimulus,
236 maintaining a response to the stimulus, recovering from the stimulus, and preparing to
237 modify future behavior after the interaction ⁵⁷). Therefore the particular gene expression
238 profile at a specific point in time is just a snapshot of a very dynamic process⁵⁸.

239 Arguably, gene lists produced by a cross-sectional transcriptomic experiment can be
240 difficult to interpret without additional controls to tease apart gene expression associated
241 with movement per se, responding to novelty, responding to any conspecific, versus
242 responding to a same-sex conspecific, etc. ⁵⁹. Another approach that is likely to be
243 insightful is to measure the time course of gene expression following a stimulus in order
244 to identify the waves of transcription associated with different components of behavioral
245 plasticity⁵⁸.

246 Increasingly, researchers are coupling comparative gene expression to the
247 examination of the mechanisms that regulate chromatin and other epigenetic
248 modifications that influence which genes are expressed and which remain silent. These
249 analyses often examine the methylation state of DNA (BS-RAD-Seq⁶⁰, meDIP, BS-
250 seq^{61,62} and pyroseq⁶³), the presence of histone-modifications and/or transcription factors
251 (ChIP-Seq)⁶⁴, or chromatin accessibility (ATAC-Seq⁶⁵).

252

253 *Genetic manipulations*

254 For some research aims, the goal is to find genes in order to understand how they
255 function to affect the biological process or trait of interest. Given the correlative nature of
256 the aforementioned methods, researchers are increasingly interested in validating their
257 results by manipulating the expression of the gene of interest in order to confirm that it
258 has a causal effect on the trait. Gene expression can be directly manipulated through
259 knock-downs that reduce function, knock-outs that eliminate function, or knock-ins that
260 replace one sequence with another or insert a novel sequence. The phenotypes of the
261 altered organism can be measured, thereby providing a direct test of functionality of
262 targeted candidate sequences. A classical technique with widespread use has been RNA
263 interference (RNAi)⁶⁶. RNAi can modify gene expression, allowing for the targeted
264 testing of pleiotropic effects, providing a clearer view of how specific genes affect the
265 strength of the correlation. RNAi can also be implemented at any life stage, but need not
266 function at every life stage. Applying RNAi in non-model species, particularly in the
267 context of manipulating brain gene expression, is not a trivial effort as it can be unreliable
268 and inefficient until protocols can be modified for new systems. More recently, the
269 CRISPR-Cas9 system has been developed for genome editing⁶⁷. It is extremely efficient
270 for gene knock-outs, and is likely to be a very accessible tool for species with amenable
271 reproductive biology, such as resilient embryos that can be manipulated. For systems
272 where this is not possible, post-mitotic CRISPR-Cas systems are a potential option for
273 localized gene manipulation^{68,69}. It also allows the insertion of novel sequences into the
274 target genome, providing a versatile tool for functional genomics; however, these knock-
275 ins are orders of magnitude less efficient than targeted deletions, and are probably a poor
276 choice for an initial causal study in a non-model system. There are, of course, limitations
277 such as difficulties in confirming the correct target sites were reached and mediating off-
278 site effects (as reviewed in Peng, Lin & Li 2016⁷⁰). Genome editing also requires one to
279 be able to rear the organism in the lab and, preferably, to affect germ-line cells, so that
280 edited genome may be passed through to subsequent generations (discussed further in
281 *The role of neuroscience*, below).

282

283 *Whole genome sequencing*

284 Understanding the genomic basis of behavioral differences often involve
285 sequencing the entirety of a species' genome. Sequencing a reference genome for a study
286 system will greatly increase the power and ease of genomic analyses described above.
287 Reference genomes enable researchers to ask more advanced questions about the
288 genomic and epigenomic variation underlying behavioral differences, such as identifying
289 regulatory regions, both cis- and trans-, involved upstream of differentially expressed
290 genes identified through RNA-Seq⁷¹. In addition, whole genome sequencing (WGS) can
291 be used to identify patterns of selection and/or divergence between closely related
292 species, populations, or behavioral types, such as relaxed selection in a large non-
293 recombining region associated with social structure in fire ants⁷², or genome
294 differentiation between diverging behavioral types of Malaria mosquitoes^{73,74}.

295 WGS does not hold the answers to all genomics questions, and as with all the
296 tools described here, requires careful consideration. Obtaining even a single, high-quality
297 reference genome can be an arduous task that is time- and resources-intensive. Genome
298 sequencing, and indeed most next generation sequencing, relies on short reads, usually
299 followed by assembly steps to yield longer sequences. One of the best measures of
300 sequence quality is coverage (or depth), i.e. how many sequence reads include a
301 particular locus. To obtain high coverage, and therefore a high-quality sequence, the
302 amount of starting material is important, including the size of the target genome and the
303 number of starting samples. Coverage will also usually be lower at polymorphic sites,
304 where high levels of heterozygosity occur, as well as in highly repetitive regions, where
305 assembly can be unreliable. Newer sequencing methods involve longer reads of single
306 molecules, which may help resolve poor assembly of highly repetitive regions. However,
307 these methods can be error-prone and require the complementary use of short-read
308 sequencing for error corrections. WGS projects, as well as genome-wide projects
309 involving assembly (such as RAD-Seq and RNA-Seq) often require tradeoffs between
310 coverage and sample size (e.g. the decision to pool samples to obtain higher quality
311 sequences, as in Reidenbach et al. 2012⁷³), where it may be beneficial to have lower
312 quality thresholds (for example reducing the target coverage from 20x to 10x), to increase
313 the available sample sizes.

314 With a known reference genome and identified candidate genetic regions, targeted
315 sequencing approaches can be used to detect genetic factors involved in repeated inter-
316 individual differences with more precision. These techniques can take advantage of PCR
317 technology to increase sequence quality without sacrificing sample size. For quantitative
318 gene expression projects, the non-linear amplification introduced by many cycles of PCR
319 can distort results, a problem that can be avoided by using simple techniques such as
320 emulsion PCR or qPCR.

321

322 **Aligning tools with questions**

323 For many questions about repeatable behavioral variation, the hope is that using
324 molecular methods will generate data that provides novel inferences about how and why
325 behavioral variation exists. This may come in the form of inductive inferences from
326 patterns- are the same genes associated with aggressiveness and exploration?-, or in
327 trying to directly connect genes to neurobiological, endocrine or physiological processes
328 that affect behavioral responses. In all of these cases, *a priori* understanding of which
329 genomic or molecular data are sufficient to test hypotheses is critical because the same
330 genomic technique may not advance every question. Each tool has a unique set of
331 strengths, weaknesses and applications to questions of interest. For example, questions
332 about within-individual change such as developmental and contextual plasticity may be
333 best-approached using tools that measure genome-wide expression (RNA-Seq). In model
334 systems, these questions could also be approached through genome and/or gene
335 expression editing techniques to manipulate the expression of genes of interest. If one or
336 several genes are suspected to regulate the correlation of multiple behaviors, for example,
337 a knockout experiment may be appealing⁷⁵. Questions more focused on between-
338 individual variation may be better served through massive parallel sequencing techniques
339 that can compare the content or structure of the genome. For example, exome capture
340 may be useful in identifying SNPs associated with different behavioral types in a GWAS
341 study, while RAD-Seq may be more useful in comparing the genomic structure between
342 behavioral types or between species via QTL mapping. **Table 2** provides example
343 hypotheses highlighting how the different tools could be applied to five fundamental
344 questions about repeatable behavioral variation, behavioral plasticity and trait co-

345 variances, thereby providing a framework to select the right tool for the question at hand.
346 This table is not intended to be a complete list of hypotheses or each tools' potential, but
347 rather a starting point for those familiarizing themselves with new tools. Below, we
348 highlight several examples to illustrate.

349 **Example #1: Use GWAS to understand why there are behavioral types.**

350 A proximal hypothesis for why there is behavioral variation among individuals
351 within natural populations is that different behavioral types of individuals have different
352 variants of a gene that influences behavior. This question was addressed in a study of the
353 silver alpine ant *Formica selysi*, which applied GWAS between social morphs to identify
354 a large Mendelian supergene associated with variation in social structure in colonies. This
355 supergene is predictive of the non-sibling queen tolerance of the colony (a key aspect of
356 the colony's personality⁷⁶); thus influencing if a colony is monogynous or polygynous⁷⁷.
357 It is plausible that once the genes relating to behavioral variation have been found,
358 genome data can be used to address whether balancing selection is maintaining the
359 genetic variation, thereby providing an ultimate answer to the same question⁷⁸.

360 **Example #2: Applying RNA-Seq to understand why individuals behave**
361 **consistently.** One possible reason why individuals might maintain a behavioral type is
362 because it's too costly to switch to a different one. According to this line of reasoning, the
363 shift between behavioral types requires dramatic transcriptional change. This is expected
364 to be costly, as it results not only in transcriptional change but also downstream
365 reorganization of signaling cascades and physiological properties, requiring both time
366 and energy. A simple prediction, then, is that there are fewer transcriptional changes
367 associated with behaviors that are highly flexible within individuals, but large
368 transcriptional changes associated with the shift between more stable behavioral types.
369 Consistent with this idea, and based on emerging evidence that it is energetically costly to
370 significantly change gene expression, sometimes with fitness consequences⁷⁹, the
371 relatively stable, permanent change in behavior of honeybee workers from nursing to
372 foraging is associated with a 39% difference in gene expression. However, changes in
373 gene expression are much lower when workers switch between less stable and more
374 flexible occupations, such as undertaker and guard^{24,80}. An alternative view is that
375 maintaining a behavioral type is similar to maintaining homeostasis. In that case, non-

376 plastic individuals, i.e. those whose behavior does not change dramatically in response to
377 the environment, might experience large fluctuations in gene expression that are related
378 to maintaining their behavioral type (Table 2). Experiments that measure gene expression
379 on a genome-wide scale can distinguish between these two hypotheses.

380 **Example #3: Applying RNA-Seq to understand why individuals differ in**
381 **behavior.** The gene expression profile of different behavioral types of individuals, e.g.
382 between genetic lines selected for high or low levels of a particular behavior, or between
383 alternative behavioral phenotypes, can be compared. Differences in expression between
384 behavioral types could be caused by genetic variation, epigenetic modifications or
385 developmental plasticity. Arguably this experimental design is better suited for answering
386 questions about the molecular causes and correlates of individual variation than
387 experiments measuring gene expression *in response to* stimuli because differences in the
388 pattern of gene expression could reflect the molecular processes involved in maintaining
389 a particular neural structure/function rather than generating a particular neurogenomic
390 state^{81,82}. This means gene expression changes may reflect suites of traits associated with
391 behavioral variation that are also often of interest to behavioral ecologists (e.g. Pace of
392 Life Syndrome)⁸³. For example, genes that are differentially expressed between
393 alternative phenotypes that differ in aggressiveness (e.g. sneaker/satellite males verses
394 territory holders) likely reflect processes involved in maintaining the molecular
395 machinery associated with morphological and life history differences between the
396 phenotypes, such as reproductive maturation⁵⁷.

397 Another tactic is to present individuals with a behavior-relevant stimulus, record
398 their behavior, measure gene expression in response to the stimulus, and include
399 individual behavior in the analysis of gene expression data. For example, in sticklebacks,
400 the expression of differentially expressed genes in response to an intruder was correlated
401 *at the individual level* with levels of aggressiveness, suggesting that differences in gene
402 expression reflect, in part, individual differences in behavior⁴⁸. Identifying genes
403 changing in expression, i.e. as an upstream promoter or downstream target, will perhaps
404 better advance our understanding of the architecture of behavioral types, as discussed
405 below. Another possibility is to compare different behavioral types in a response
406 experiment to the same control and to ask if there are more differentially expressed genes

407 between one extreme behavioral type and the unexposed control of some type versus
408 another behavioral type and its control?

409 **Example #4: Applying RNA-Seq to understand why traits are correlated.**

410 When individual differences in behavior are correlated in response to different
411 ecologically-relevant stimuli (e.g. a potential mate, competitor, predator, novel
412 environment, etc.), genes that are differentially expressed in response to both stimuli are
413 plausible molecular causes or correlates of the behavioral syndrome. Correlated
414 behaviors can be expressed through modulation of the expression of the same causative
415 loci in different contexts. Comparative gene expression analyses involving individuals
416 responding to different cues can reveal the role of up- and down-regulation of the same
417 genes in different contexts. For example, the expression of behavioral genes in
418 stickleback fish change both in response to courtship, and in response to aggression;
419 albeit in different directions. This suggests that such genes are involved in both
420 aggressive and courtship behaviors, causing behavioral traits to be correlated⁸⁴.

421 **Example #5: Using gene association studies to understand why traits are**
422 **correlated.** One possible explanation for behavioral syndromes is that different behaviors
423 are influenced by the same genes, or set of physically linked loci. If this is the case, then
424 the different behavioral traits will map to the same genomic region(s) in gene association
425 studies. For example, the close proximity of two QTLs participating in male display
426 behavior and female preference in *Lapaula* crickets explains the correlation of both of
427 these traits³¹. At the individual level, correlated traits of white-throated sparrows involved
428 in parental care, plumage, and aggression were linked to co-expression of genes located
429 in a chromosomal inversion⁸⁵.

430

431 Of course, no one tool is the magic bullet to resolve the persistent questions in
432 repeatable behavioral variation research. Instead, the most important insights, as well as
433 the most successful research avenues are likely to come when tools with complementary
434 strengths are integrated together (Box 2). For example, by integrating RNA-Seq and ChiP
435 seq, Bukhari and colleagues demonstrated rapid and dramatic epigenomic plasticity in
436 response to social interactions in three-spined sticklebacks. This was facilitated by

437 integrating brain gene expression data with a transcriptional regulatory network, and
438 linking gene expression to changes in chromatin accessibility (Figure 1)⁵⁸.

439

440 **Inferring the function of genes related to behavior**

441 Twenty years ago, sequencing the human genome was one of the most ambitious
442 scientific endeavors ever attempted. However, genomic technology has advanced quickly
443 and has rapidly outpaced our computational capabilities, creating challenges in
444 interpreting genomic data. Yet, there are strategies that work broadly and are applicable
445 to research in repeatable behavioral variation and behavior generally, such as functional
446 annotation and gene classification. This is only a subsampling of the analytical tools
447 (most of which are covered in a quality genomic sciences primer; e.g. Gibson & Muse, A
448 Primer of Genome Science⁸⁶), and considerations for inferring gene function (see also
449 Rittschof and Robinson⁵⁰ for a more thorough discussion of these topics).

450 A primary goal of any genome-sequencing project is to classify genes into
451 putative functional families. This allows for necessary comparisons, perhaps to look for
452 genes overrepresented or underrepresented as compared to other genomes. While
453 alignment (where a reference genome is available) or assembly (where one is not) may be
454 a first step in functional annotation, this method is insufficient and error prone, meaning
455 further steps are required⁸⁷. Using software such as BLAST, amino acid similarity to
456 previously annotated genes can be compared. However, it is not uncommon for one-third
457 to one half of the genome to remain unclassified after such an analysis. Numerous
458 databases that are often discipline-specific have been established to classify these protein
459 domains. Currently, however, no such database exists for behavioral genomic data.

460 Annotation based on molecular function is insufficient to describe or predict
461 biological function. An annotation cannot take directly into account neo-
462 functionalization, where the physiological function of a gene has evolved, or where
463 phenome-level traits have split from one gene to several⁸⁶. Yet, there is still highly
464 conserved gene function across most animal taxa, meaning that understanding the
465 ontology of genes still holds value⁵⁷ (but see the phenolog concept⁸⁸). Projects such as the
466 Gene Ontology (GO) Consortium (geneontology.com) work to try and find unifying

467 functions of genes and gene products across eukaryotes⁸⁹. As cell biologists and
468 physiologists have pioneered much of the GO work, the annotations are biased as such.
469 This can make direct inferences about behavioral variation difficult. While smaller than
470 the GOC, one resource of particular value to behavior researchers may be
471 Geneweaver.org, which includes the functional effects on behavior when available and
472 has consolidated much of the published behavioral genomics work⁹⁰.

473 The dearth of resources for relating the function of genes to processes of interest
474 to behavioral ecologists is apparent. This results in some of the ‘skepticism’ about what
475 insights come from behavioral variation studies that yield lists of up vs. down regulated
476 genes, but no functional significance of these gene expression patterns (though the
477 pattern is sometimes important irrespective of the gene identities⁵), or no clear next step.
478 This problem may be resolved by deploying other strategies or applying other tools to
479 further explore genes of interest. Simply knowing the number of genes changing their
480 expression may not be inherently helpful. It may seem an obvious prediction that big
481 switches between behaviors are a result of big transcriptional changes. Perhaps this may
482 be proposed as a hypothesis for why individuals stay consistent- because big switches are
483 mechanistically difficult. However, this may be an over simplification, as the number or
484 size of a transcriptional change may not reflect the ultimate ‘cost’ to the organism.

485 Creating databases with behavior – centric protein domains or gene ontologies is
486 an alluring, but a potentially impractical path for pioneering behavioral ecologists.
487 However, understanding repeatable behavioral variation is inherently integrative, with
488 researchers putting forth a significant number of hypotheses for why this variation has
489 emerged and been maintained. Many of these hypotheses holistically incorporate
490 different trait types; for example, it has been suggested that physiology (fast-slow
491 physiology^{91,92}), life-history strategy (pace-of-life hypothesis⁸³), speed-accuracy trade-
492 offs in cognitive differences⁹³ and variation in immune function⁹⁴ may drive observed
493 behavioral variation. Using a technique such as a functional enrichment analysis could be
494 used to further this integration. For example, if gene ontology terms are found to be
495 related to metabolism, neurotransmission or immunity, this may offer support for
496 particular hypotheses about behavioral variation. A practical approach may be to better

497 integrate with genomicists or other researchers already developing gene ontology
498 databases.

499

500 **The role of neuroscience: linking genome function to behavioral variation through** 501 **the brain**

502 The path from genome to phenome passes through the structure and function of
503 the nervous system, where changes in gene expression influence the development of
504 circuit connectivity or transiently modulate cellular properties. Because the brain is
505 characterized by its exquisite heterogeneity of cell types, a satisfying link between
506 genetic mechanisms and behavioral outcomes requires attending to differences in specific
507 brain regions and circuits. As a result, the “where” and “when” of gene expression are
508 critical considerations. While this seems daunting to most behavioral ecologists, there are
509 good suggestions for how best to incorporate neuroscience when considering the
510 biological mechanisms that contribute to repeatable behavioral variation.

511 One of the main reasons to incorporate neuroscience into studies of behavioral
512 variation is to improve the power of genomic approaches by focusing efforts on relevant
513 circuits and brain regions. Performing an RNAseq study with an entire brain could reveal
514 important differences (Table 2), but because the sequencing will include a majority of
515 transcripts from brain regions unrelated to the behavior of interest, the effects of
516 important genes may be masked, or may require an increase in sequencing effort to
517 detect. There are well-known circuits for most dimensions of behavior that interest
518 behavioral variation researchers, including aggression, boldness, and energy balance, and
519 the last decade has witnessed substantial advances in identifying the homologs of brain
520 regions across vertebrates⁹⁵⁻⁹⁷. Knowing these circuits can assist in our understanding of
521 trait correlations, as well. For example, if the same nodes within these circuits are
522 involved in multiple behaviors, correlations between traits might emerge from variation
523 in gene expression across these common neural structures^{98,99}.

524 Although the *a priori* choice of brain regions based on homology is powerful and
525 general, an alternative approach is to use an unbiased method to identify brain regions
526 that differ in function between individuals with alternative phenotypes. One simple, but
527 neglected method is to examine the metabolic activity of brain regions by staining for

528 cytochrome oxidase¹⁰⁰, the rate-limiting enzyme in oxidative phosphorylation that
529 changes in response to use that spans days or weeks – a time scale well suited to
530 behavioral variation research (Rittschof et al., in review). Brain regions exhibiting
531 differences in metabolism could be useful targets for subsequent studies of the
532 transcriptome or epigenome.

533 Another, more common approach is to examine the expression of specific genes
534 that are expressed in response to recent neural activity, known as “immediate early genes
535 (IEGs).” By evoking a specific behavior from an animal, relevant brain regions become
536 activated and, with modest delay, express immediate early genes. However, commercial
537 antibodies for detecting IEG gene products tend to transfer poorly to non-mammalian
538 species (though this complication can be circumvented by using mRNA *in situ*
539 hybridization¹⁰¹). Thus, a promising new approach is to examine the transient
540 phosphorylation of actively translating ribosomes¹⁰², marks that are enriched by neural
541 activity and highly conserved across taxa. Sequencing the mRNA being actively
542 translated by these phosphorylated ribosomes also allows the researcher to identify
543 neurons of interest, for instance via their expression of specific neurotransmitter-related
544 genes. Though still preliminary in its application, this tool may be useful for researchers
545 studying behavioral variation.

546 Once specific genes and brain regions have been identified, the next logical step is
547 to manipulate gene expression, such as using CRISPR/Cas9 to create knock-outs and
548 observe their behavioral consequences^{103–106}. However, the absence of a gene product
549 throughout the animal’s life provides a relatively poor model of naturally-occurring
550 genetic or transcriptional variation, and biological compensation can mask the effects of
551 some gene knockouts. Thus, more relevant approaches may be ones in which levels of
552 gene transcription can be manipulated in a regionally- and temporally-refined manner
553 within the nervous system. RNAi¹⁰⁷, modified Cas9-fusion proteins (see Table 2)^{105,108},
554 and CRISPR-mediated site-specific epigenetic modifications¹⁰⁹ represent tools for
555 achieving localized manipulations of gene function. In the lab, these can be introduced
556 via replication-deficient viral vectors. However, transitioning these technologies to the
557 field may require additional safety considerations.

558 The decision to incorporate neuroscience and mechanistic studies does require
559 consideration of what will be gained from doing so. In particular, doing so may help
560 reveal whether there are general principles that explain the neural mechanisms underlying
561 repeatable behavioral variation across species. Additionally, focusing on key brain areas
562 for gene expression may provide cleaner data for more targeted hypothesis testing, as
563 well as a better understanding of how genetic variation exerts its effect on behavioral
564 phenotype.

565 **Is behavioral genomics right for you?**

566 Entering the world of behavioral genomics comes with a considerable set of
567 challenges and considerations. For example, organisms with large genomes, that are
568 polyploid and have a large number of repetitive elements are likely to pose challenges for
569 studies that require an assembled genome, and certain organisms are going to be easier to
570 manipulate than others (Box 1). Moreover, it is worth carefully considering whether the
571 benefits of using these tools are likely to outweigh their considerable costs. As argued in
572 this paper, we clearly think the answer is “yes”, and that there are apparent opportunities
573 for research investigating repeatable behavioral variation to benefit from incorporating
574 modern genomic techniques (Box 2). We hope that this is an appealing direction to many,
575 with the possibility of pioneering new analytical methods and taking a leadership position
576 in directing this field forward.

577

578 **Figure Legends:**

579 **Table 1:** Logistic constraints associated with popular high-throughput and experimental
580 techniques.

581

582 **Table 2:** Applying tools to questions. Shown are possible ways to apply tools to five
583 outstanding questions about repeatable behavioral variation. Specific tools may have
584 limitations making them unsuitable for specific taxa or approaches (see Table 1 and text).
585 While methodologically very different tools, the application of RAD-seq data is similar
586 to that of Genotyping-by-Sequencing data (and of any other genotyping method

587 involving random markers in the genome), therefore only one column was included
588 describing the application of both.

589

590 **Figure 1:** A flowchart of a hypothetical study emphasizing the benefit of integrating
591 multiple tools to understand repeatable behavioral variation; in this case, combining
592 RNA-Seq and ChIP-Seq to investigate differences in behavioral plasticity. The orange
593 arrow and boxes represent what can be inferred from a ChIP-Seq protocol alone; blue
594 arrows and boxes represent what can be inferred from a RNA-Seq protocol alone. Golden
595 arrows and boxes highlight what can be inferred by combining both tools to obtain
596 complementary information. DEG = differentially expressed genes, TF = transcription
597 factor, ASTRIX = Analyzing subsets of Transcriptional Regulators Influencing
598 eXpression. Inspired by methods utilized by Bukhari and colleagues⁵⁸.

599

600

601

602 **Box 1: Perks and perils of the model organism**

603 Though relatively loosely defined¹¹⁰, model organisms are generally systems with traits
604 predisposing them to be tractable for experimental manipulation. Such traits may include
605 readily reproducing in laboratory settings with relatively short generation times and large
606 clutch or litter sizes, robust embryos that can survive manipulation and share important
607 genes across multiple taxa (e.g., with humans). To geneticists and many evolutionary
608 biologists, these models may be little more than functional bags of chromosomes. Yet
609 many model species have highly dynamic and complex behaviors that are of interest to
610 behavioral ecologists focused on repeatable behavioral variation. For example, in
611 *Drosophila melanogaster*, the social context can affect both the aggressive behavior and
612 fitness of males¹¹¹, zebra fish (*Danio rerio*) have innate variation in their response to
613 threats¹¹² and honey bee workers show some of the most predictable and well understood
614 behavioral plasticity known^{24,113}. Additionally, some model organisms have been useful
615 in discovering well-conserved genetic “toolkits”, for complex traits such as social
616 behavior²³. Of course, care must be taken to account for potential behavioral changes

617 associated with many generations of adaptation to laboratory conditions. It may be
618 challenging to interpret adaptive significance of model organism behavior compared to
619 'wild' systems. However, researchers interested in causal mechanisms should consider
620 focusing their attention on such promising organisms. Robust techniques and
621 methodologies have already been developed and genomes are well annotated with easily
622 searchable gene ontology databases. Through decades of neurobiology, candidate
623 systems and genes of interest for behaviors and behavioral (and synaptic) plasticity are
624 already well described. For a behavioral variation researcher wanting to venture into
625 genomics, perhaps to test a new hypothesis, model organisms may be the easiest point of
626 entry. However, as in any field there are costs to this approach, such as limiting the
627 diversity of taxa seen in the field, and model organisms do not necessarily have the traits
628 of interest to many behavioral ecologists. It may also not be the right direction for
629 students who want to develop their own system to utilize over the course of their career.
630 Yet researchers should not lightly overlook the value available in a model system.

631

632

633

634

Box 2: Integrating tools

635

636

637

638

639

640

641

As tools increase in popularity and accessibility, it will become increasingly possible to use them in complementary ways. This is attractive, because each tool has strengths and weaknesses, and investigations of the toughest questions about repeatable individual variation at the molecular level will best be served by the application of multiple genomic tools in creative and complementary ways. Here we offer examples of how studies of behavioral variation are likely to benefit from the simultaneous application of multiple tools.

642

643

644

645

646

647

Example #1: Integrating RNA-Seq and GWAS/QTL to find genes related to behavioral variation. One of the main drawbacks to gene association studies is the large sample size needed in order to narrow the window harboring key genetic variants. An obvious way to reduce the search space and hence improve power to detect loci is to prioritize regions of the genome that harbor genes that are differentially expressed, based on results from RNA-Seq studies.

648 **Example #2:** Integrating RNA-Seq and epigenomics (ChIP-Seq, ATAC-Seq,
649 methylation profiling) to understand behavioral plasticity. Many of the outstanding
650 questions about repeatable behavioral variation have to do with behavioral plasticity.
651 Transcriptomic profiling is especially well suited for investigating behavioral plasticity at
652 the molecular level, but our questions are increasingly focused on upstream regulators of
653 transcriptional plasticity, especially insofar as they might tell us about the causes of
654 variation in plasticity. If we can identify key regulatory elements that govern changes in
655 gene expression (e.g. histone modifications, transcription factor binding sites,
656 methylation and chromatin accessibility), then we can start asking questions about
657 genetic variation in those elements, which might be related to individual differences in
658 plasticity.

659 **Example #3:** Integrating gene association studies with epigenomics to explore
660 constraints on plasticity. Individuals may be behaviorally constrained if their behavioral
661 type is genetically or epigenetically determined. Genetic markers generated via
662 genotyping-by-sequencing or RAD-Seq can be used to perform a GWAS or to identify
663 QTLs that may be associated with a specific behavioral type, identifying genetic regions
664 in linkage disequilibrium with causative alleles involved in constraining plasticity, as
665 discussed above. On the other hand, epigenetic modifications, such as histone
666 modifications or methylation differences, can be identified using ChIP-seq, RRBS,
667 WGBS or pyrosequencing and have been shown to affect behavior⁶². Functional tests can
668 be used to confirm results. For example, histone modifications in carpenter ants have
669 been shown to directly affect foraging behavior of workers. If these modifications are
670 altered, the behavior of workers is also altered, suggesting a causal link between
671 chromatin state and constraints on behavioral plasticity¹¹⁴.

672

673

674 **Acknowledgements:** The workshop that led to this series of papers was funded by the
675 NSF (NSF-IOS 1623898; PI: AM Bell), the NSF Sociogenomics RCN and the Carl R.
676 Woese Institute for Genomic Biology at the University of Illinois Urbana Champaign.
677 We wish to thank other workshop participants for their feedback in the development of
678 these ideas, and comments on drafts of the manuscript.

679

680 **References:**

- 681 1. Dingemanse, N.J., Both, C., Drent, P.J., van Oers, K. & van Noordwijk, A.J.
682 Repeatability and heritability of exploratory behaviour in great tits from the wild.
683 *Anim. Behav.* **64**, 929–938 (2002).
- 684 2. Sih, A., Bell, A. & Johnson, J.C. Behavioral syndromes: an ecological and
685 evolutionary overview. *Trends Ecol. Evol.* **19**, 372–378 (2004).
- 686 3. Pruitt, J.N. & Keiser, C.N. The personality types of key catalytic individuals shape
687 colonies' collective behaviour and success. *Anim. Behav.* **93**, 87–95 (2014).
- 688 4. Bengtson, S.E. & Dornhaus, A. Be meek or be bold? A colony-level behavioural
689 syndrome in ants. *Proc. R. Soc. B Biol. Sci.* **281**, 20140518 (2014).
- 690 5. Rittschof, C.C. & Robinson, G.E. Genomics: moving behavioural ecology beyond the
691 phenotypic gambit. *Anim. Behav.* **92**, 263–270 (2014).
- 692 6. Ramirez-Esquivel, F., Leitner, N.E., Zeil, J. & Narendra, A. The sensory arrays of the
693 ant, *Temnothorax rugatulus*. *Arthropod Struct. Dev.* (2017).
694 doi:10.1016/j.asd.2017.03.005
- 695 7. Oers, K. van & Mueller, J.C. Evolutionary genomics of animal personality. *Philos.*
696 *Trans. R. Soc. Lond. B Biol. Sci.* **365**, 3991–4000 (2010).
- 697 8. Bell, A.M. & Dochtermann, N.A. Integrating molecular mechanisms into quantitative
698 genetics to understand consistent individual differences in behavior. *Curr. Opin.*
699 *Behav. Sci.* **6**, 111–114 (2015).
- 700 9. Briffa, M. & Sneddon, L.U. Proximate mechanisms of animal personality among-
701 individual behavioural variation in animals. *Behaviour* **153**, 1509–1515 (2016).

- 702 10. Mackay, T.F.C. Epistasis and quantitative traits: using model organisms to study
703 gene-gene interactions. *Nat. Rev. Genet.* **15**, 22–33 (2014).
- 704 11. Stamps, J.A. & Biro, P.A. Personality and individual differences in plasticity. *Curr.*
705 *Opin. Behav. Sci.* **12**, 18–23 (2016).
- 706 12. Dingemanse, N.J. & Wolf, M. Between-individual differences in behavioural
707 plasticity within populations: causes and consequences. *Anim. Behav.* **85**, 1031–1039
708 (2013).
- 709 13. Grafen, A. Natural selection, kin selection and group selection. in *Behavioural*
710 *ecology, 2nd edition* (eds. Krebs, J. & Davies, N.) 62–84 (Blackwell Scientific
711 Publications, Oxford, 1984).
- 712 14. Bateson, P. & Laland, K.N. Tinbergen’s four questions: an appreciation and an
713 update. *Trends Ecol. Evol.* **28**, 712–718 (2013).
- 714 15. Stamps, J. Behavioural processes affecting development: Tinbergen’s fourth question
715 comes of age. *Anim. Behav.* **66**, 1–13 (2003).
- 716 16. Travisano, M. & Shaw, R.G. Lost in the map. *Evolution* **67**, 305–314 (2013).
- 717 17. Paaby, A.B. & Rockman, M.V. The many faces of pleiotropy. *Trends Genet.* **29**, 66–
718 73 (2013).
- 719 18. Zuk, M. & Balenger, S.L. Behavioral ecology and genomics: new directions, or just a
720 more detailed map? *Behav. Ecol.* **25**, 1277–1282 (2014).
- 721 19. Fitzpatrick, M.J. *et al.* Candidate genes for behavioural ecology. *Trends Ecol. Evol.*
722 **20**, 96–104 (2005).
- 723 20. West-Eberhard, M.J. *Developmental Plasticity and Evolution*. (Oxford University
724 Press, 2003).

- 725 21. Saltz, J.B., Hessel, F.C. & Kelly, M.W. Trait correlations in the genomics era. *Trends*
726 *Ecol. Evol.* **32**, 279–290 (2017).
- 727 22. Sabeti, P.C. *et al.* Genome-wide detection and characterization of positive selection
728 in human populations. *Nature* **449**, 913–918 (2007).
- 729 23. Rittschof, C.C. *et al.* Neuromolecular responses to social challenge: Common
730 mechanisms across mouse, stickleback fish, and honey bee. *Proc. Natl. Acad. Sci.*
731 201420369 (2014). doi:10.1073/pnas.1420369111
- 732 24. Whitfield, C.W., Cziko, A.M. & Robinson, G.E. Gene expression profiles in the brain
733 predict behavior in individual honey bees. *Science* **302**, 296–299 (2003).
- 734 25. Bendesky, A. *et al.* The genetic basis of parental care evolution in monogamous
735 mice. *Nature* **544**, 434–439 (2017).
- 736 26. Stamps, J.A. & Frankenhuis, W.E. Bayesian models of development. *Trends Ecol.*
737 *Evol.* **31**, 260–268 (2016).
- 738 27. Sih, A. *et al.* Animal personality and state–behaviour feedbacks: a review and guide
739 for empiricists. *Trends Ecol. Evol.* **30**, 50–60 (2015).
- 740 28. Snell-Rood, E.C. An overview of the evolutionary causes and consequences of
741 behavioural plasticity. *Anim. Behav.* **85**, 1004–1011 (2013).
- 742 29. Hirschhorn, J.N. & Daly, M.J. Genome-wide association studies for common
743 diseases and complex traits. *Nat. Rev. Genet.* **6**, 95–108 (2005).
- 744 30. Slate, J. From beavis to beak color: a simulation study to examine how much QTL
745 mapping can reveal about the genetic architecture of quantitative traits. *Evolution* **67**,
746 1251–1262 (2013).

- 747 31. Shaw, K.L. & Lesnick, S.C. Genomic linkage of male song and female acoustic
748 preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci.* **106**,
749 9737–9742 (2009).
- 750 32. Lynch, M. & Walsh, B. Genetics and analysis of quantitative traits. *Genet. Anal.*
751 *Quant. Traits* (1997).
- 752 33. Küpper, C. *et al.* A supergene determines highly divergent male reproductive morphs
753 in the ruff. *Nat. Genet.* **48**, 79–83 (2016).
- 754 34. Berens, A.J., Hunt, J.H. & Toth, A.L. Comparative transcriptomics of convergent
755 evolution: different genes but conserved pathways underlie caste phenotypes across
756 lineages of eusocial insects. *Mol. Biol. Evol.* **32**, 690–703 (2015).
- 757 35. Anholt, R.R.H. *et al.* The genetic architecture of odor-guided behavior in *Drosophila*:
758 epistasis and the transcriptome. *Nat. Genet.* **35**, 180–184 (2003).
- 759 36. Bell, A.M. & Robinson, G.E. Behavior and the dynamic genome. *Science* **332**, 1161–
760 1162 (2011).
- 761 37. Lawniczak, M.K. & Begun, D.J. A genome-wide analysis of courting and mating
762 responses in *Drosophila melanogaster* females. *Genome* **47**, 900–910 (2004).
- 763 38. Mack, P.D., Kapelnikov, A., Heifetz, Y. & Bender, M. Mating-responsive genes in
764 reproductive tissues of female *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **103**,
765 10358–10363 (2006).
- 766 39. Carney, G.E. A rapid genome-wide response to *Drosophila melanogaster* social
767 interactions. *BMC Genomics* **8**, 288 (2007).
- 768 40. Cummings, M.E. *et al.* Sexual and social stimuli elicit rapid and contrasting genomic
769 responses. *Proc. R. Soc. Lond. B Biol. Sci.* **275**, 393–402 (2008).

- 770 41. McGraw, L.A., Clark, A.G. & Wolfner, M.F. Post-mating gene expression profiles of
771 female *Drosophila melanogaster* in response to time and to four male accessory
772 gland proteins. *Genetics* **179**, 1395–1408 (2008).
- 773 42. Fraser, B.A., Janowitz, I., Thairu, M., Travis, J. & Hughes, K.A. Phenotypic and
774 genomic plasticity of alternative male reproductive tactics in sailfin mollies. *Proc. R.*
775 *Soc. Lond. B Biol. Sci.* **281**, 20132310 (2014).
- 776 43. Mori, T. *et al.* Genetic basis of phenotypic plasticity for predator-induced
777 morphological defenses in anuran tadpole, *Rana pirica*, using cDNA subtraction and
778 microarray analysis. *Biochem. Biophys. Res. Commun.* **330**, 1138–1145 (2005).
- 779 44. Sanogo, Y.O., Hankison, S., Band, M., Obregon, A. & Bell, A.M. Brain
780 transcriptomic response of threespine sticklebacks to cues of a predator. *Brain.*
781 *Behav. Evol.* **77**, 270–285 (2011).
- 782 45. Becks, L., Ellner, S.P., Jones, L.E. & Hairston, N.G. The functional genomics of an
783 eco-evolutionary feedback loop: linking gene expression, trait evolution, and
784 community dynamics. *Ecol. Lett.* **15**, 492–501 (2012).
- 785 46. Lavergne, S.G., McGowan, P.O., Krebs, C.J. & Boonstra, R. Impact of high
786 predation risk on genome-wide hippocampal gene expression in snowshoe hares.
787 *Oecologia* **176**, 613–624 (2014).
- 788 47. Alaux, C. *et al.* Honey bee aggression supports a link between gene regulation and
789 behavioral evolution. *Proc. Natl. Acad. Sci.* **106**, 15400–15405 (2009).
- 790 48. Sanogo, Y.O., Band, M., Blatti, C., Sinha, S. & Bell, A.M. Transcriptional regulation
791 of brain gene expression in response to a territorial intrusion. in *Proc. R. Soc. B*
792 rspb20122087 (The Royal Society, 2012).

- 793 49. Rittschof, C.C. & Robinson, G.E. Manipulation of colony environment modulates
794 honey bee aggression and brain gene expression. *Genes Brain Behav.* **12**, 802–811
795 (2013).
- 796 50. Rittschof, C.C. & Robinson, G.E. Behavioral genetic toolkits: toward the
797 evolutionary origins of complex phenotypes. in *Current Topics in Developmental*
798 *Biology* **119**, 157–204 (Academic Press, 2016).
- 799 51. Jandt, J.M., Thomson, J.L., Geffre, A.C. & Toth, A.L. Lab rearing environment
800 perturbs social traits: a case study with *Polistes* wasps. *Behav. Ecol.* **26**, 1274–1284
801 (2015).
- 802 52. Tylee, D.S., Kawaguchi, D.M. & Glatt, S.J. On the outside, looking in: a review and
803 evaluation of the comparability of blood and brain ‘-omes’. *Am. J. Med. Genet. B*
804 *Neuropsychiatr. Genet.* **162**, 595–603 (2013).
- 805 53. Nikolova, Y.S. & Hariri, A.R. Can we observe epigenetic effects on human brain
806 function? *Trends Cogn. Sci.* **19**, 366–373 (2015).
- 807 54. Hannon, E., Lunnon, K., Schalkwyk, L. & Mill, J. Interindividual methylomic
808 variation across blood, cortex, and cerebellum: implications for epigenetic studies of
809 neurological and neuropsychiatric phenotypes. *Epigenetics* **10**, 1024–1032 (2015).
- 810 55. Derks, M.F.L. *et al.* Gene and transposable element methylation in great tit (*Parus*
811 *major*) brain and blood. *BMC Genomics* **17**, 332 (2016).
- 812 56. Cullinan, W.E., Herman, J.P., Battaglia, D.F., Akil, H. & Watson, S.J. Pattern and
813 time course of immediate early gene expression in rat brain following acute stress.
814 *Neuroscience* **64**, 477–505 (1995).

- 815 57. Aubin-Horth, N. & Renn, S.C.P. Genomic reaction norms: using integrative biology
816 to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **18**, 3763–
817 3780 (2009).
- 818 58. Bukhari, S.A. *et al.* Temporal dynamics of neurogenomic plasticity in response to
819 social interactions in male three spined sticklebacks. *PLoS Genetics* (in press).
- 820 59. Bell, A.M., Bukhari, S.A. & Sanogo, Y.O. Natural variation in brain gene expression
821 profiles of aggressive and nonaggressive individual sticklebacks. *Behaviour* **153**,
822 1723–1743 (2016).
- 823 60. Trucchi, E. *et al.* BsRADseq: screening DNA methylation in natural populations of
824 non-model species. *Mol. Ecol.* **25**, 1697–1713 (2016).
- 825 61. Glastad, K.M., Gokhale, K., Liebig, J. & Goodisman, M.A.D. The caste- and sex-
826 specific DNA methylome of the termite *Zootermopsis nevadensis*. *Sci. Rep.* **6**,
827 (2016).
- 828 62. Verhulst, E.C. *et al.* Evidence from pyrosequencing indicates that natural variation in
829 animal personality is associated with DRD4 DNA methylation. *Mol. Ecol.* **8**, 1801–
830 1811 (2016).
- 831 63. Laine, V.N. *et al.* Evolutionary signals of selection on cognition from the great tit
832 genome and methylome. *Nat. Commun.* **7**, ncomms10474 (2016).
- 833 64. Cronican, A.A. *et al.* Genome-wide alteration of histone H3K9 acetylation pattern in
834 mouse offspring prenatally exposed to arsenic. *PLOS ONE* **8**, e53478 (2013).
- 835 65. Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y. & Greenleaf, W.J.
836 Transposition of native chromatin for fast and sensitive epigenomic profiling of open

- 837 chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* **10**, 1213–
838 1218 (2013).
- 839 66. Hunter, C.P. Genetics: A touch of elegance with RNAi. *Curr. Biol.* **9**, R440–R442
840 (1999).
- 841 67. Hsu, P.D., Lander, E.S. & Zhang, F. Development and applications of CRISPR-Cas9
842 for genome engineering. *Cell* **157**, 1262–1278 (2014).
- 843 68. Straub, C., Granger, A.J., Saulnier, J.L. & Sabatini, B.L. CRISPR/Cas9-mediated
844 gene knock-down in post-mitotic neurons. *PLOS ONE* **9**, e105584 (2014).
- 845 69. Swiech, L. *et al.* In vivo interrogation of gene function in the mammalian brain using
846 CRISPR-Cas9. *Nat. Biotechnol.* **33**, 102–106 (2015).
- 847 70. Peng, R., Lin, G. & Li, J. Potential pitfalls of CRISPR/Cas9-mediated genome
848 editing. *FEBS J.* **283**, 1218–1231 (2016).
- 849 71. Chandrasekaran, S. *et al.* Behavior-specific changes in transcriptional modules lead
850 to distinct and predictable neurogenomic states. *Proc. Natl. Acad. Sci.* **108**, 18020–
851 18025 (2011).
- 852 72. Wang, J. *et al.* A Y-like social chromosome causes alternative colony organization in
853 fire ants. *Nature* **493**, 664–668 (2013).
- 854 73. Reidenbach, K.R. *et al.* Patterns of genomic differentiation between ecologically
855 differentiated M and S forms of *Anopheles gambiae* in West and Central Africa.
856 *Genome Biol. Evol.* **4**, 1202–1212 (2012).
- 857 74. Lawniczak, M.K.N. *et al.* Widespread divergence between incipient *Anopheles*
858 *gambiae* species revealed by whole genome sequences. *Science* **330**, 512–514 (2010).

- 859 75. Chalfin, L. *et al.* Mapping ecologically relevant social behaviours by gene knockout
860 in wild mice. *Nat. Commun.* **5**, 4569 (2014).
- 861 76. Jandt, J.M. *et al.* Behavioural syndromes and social insects: personality at multiple
862 levels. *Biol. Rev.* **89**, 48–67 (2014).
- 863 77. Purcell, J., Brelsford, A., Wurm, Y., Perrin, N. & Chapuisat, M. Convergent genetic
864 architecture underlies social organization in ants. *Curr. Biol.* **24**, 2728–2732 (2014).
- 865 78. Rausher, M.D. & Delph, L.F. When does understanding phenotypic evolution require
866 identification of the underlying genes? *Evolution* **69**, 1655–1664 (2015).
- 867 79. Lang, G.I., Murray, A.W. & Botstein, D. The cost of gene expression underlies a
868 fitness trade-off in yeast. *Proc. Natl. Acad. Sci.* **106**, 5755–5760 (2009).
- 869 80. Cash, A.C., Whitfield, C.W., Ismail, N. & Robinson, G.E. Behavior and the limits of
870 genomic plasticity: power and replicability in microarray analysis of honeybee
871 brains. *Genes Brain Behav.* **4**, 267–271 (2005).
- 872 81. Zayed, A. & Robinson, G.E. Understanding the relationship between brain gene
873 expression and social behavior: lessons from the honey bee. *Annu. Rev. Genet.* **46**,
874 591–615 (2012).
- 875 82. Cardoso, S.D., Teles, M.C. & Oliveira, R.F. Neurogenomic mechanisms of social
876 plasticity. *J. Exp. Biol.* **218**, 140–149 (2015).
- 877 83. Réale, D. *et al.* Personality and the emergence of the pace-of-life syndrome concept
878 at the population level. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**, 4051–4063
879 (2010).

- 880 84. Sanogo, Y.O. & Bell, A.M. Molecular mechanisms and the conflict between
881 courtship and aggression in three-spined sticklebacks. *Mol. Ecol.* **25**, 4368–4376
882 (2016).
- 883 85. Zinzow-Kramer, W.M. *et al.* Genes located in a chromosomal inversion are
884 correlated with territorial song in white-throated sparrows. *Genes Brain Behav.* **14**,
885 641–654 (2015).
- 886 86. Gibson, G. & Muse, S.V. *A Primer of Genome Science*. (Sinauer Associates, 2009).
- 887 87. Flicek, P. & Birney, E. Sense from sequence reads: methods for alignment and
888 assembly. *Nat. Methods* **6**, S6–S12 (2009).
- 889 88. McGary, K.L. *et al.* Systematic discovery of nonobvious human disease models
890 through orthologous phenotypes. *Proc. Natl. Acad. Sci.* **107**, 6544–6549 (2010).
- 891 89. Ashburner, M. *et al.* Gene Ontology: tool for the unification of biology. *Nat. Genet.*
892 **25**, 25–29 (2000).
- 893 90. Baker, E. J., Jay, J.J., Bubier, J.A., Langston, M.A. & Chesler, E.J. GeneWeaver: a
894 web-based system for integrative functional genomics. *Nucleic Acids Res.* **40**,
895 D1067–D1076 (2012).
- 896 91. Biro, P.A. & Stamps, J.A. Do consistent individual differences in metabolic rate
897 promote consistent individual differences in behavior? *Trends Ecol. Evol.* **25**, 653–
898 659 (2010).
- 899 92. Biro, P.A. & Stamps, J.A. Are animal personality traits linked to life-history
900 productivity? *Trends Ecol. Evol.* **23**, 361–368 (2008).

- 901 93. Sih, A. & Giudice, M.D. Linking behavioural syndromes and cognition: a
902 behavioural ecology perspective. *Philos. Trans. R. Soc. B Biol. Sci.* **367**, 2762–2772
903 (2012).
- 904 94. Tieleman, B.I., Williams, J.B., Ricklefs, R.E. & Klasing, K.C. Constitutive innate
905 immunity is a component of the pace-of-life syndrome in tropical birds. *Proc. R. Soc.*
906 *Lond. B Biol. Sci.* **272**, 1715–1720 (2005).
- 907 95. O’Connell, L.A. & Hofmann, H.A. The vertebrate mesolimbic reward system and
908 social behavior network: a comparative synthesis. *J. Comp. Neurol.* **519**, 3599–3639
909 (2011).
- 910 96. Goodson, J.L. The vertebrate social behavior network: evolutionary themes and
911 variations. *Horm. Behav.* **48**, 11–22 (2005).
- 912 97. Newman, S.W. The medial extended amygdala in male reproductive behavior, a node
913 in the mammalian social behavior network. *Ann. N. Y. Acad. Sci.* **877**, 242–257
914 (1999).
- 915 98. Newman, M. The structure and function of complex networks. *SIAM Rev.* **45**, 167–
916 256 (2003).
- 917 99. Newman, M.E J. & Clauset, A. Structure and inference in annotated networks. *Nat.*
918 *Commun.* **7**, 11863 (2016).
- 919 100. Wong-Riley, M. Changes in the visual system of monocularly sutured or
920 enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Res.*
921 **171**, 11–28 (1979).

- 922 101. Laiho, J.E. *et al.* Relative sensitivity of immunohistochemistry, multiple reaction
923 monitoring mass spectrometry, in situ hybridization and PCR to detect
924 Coxsackievirus B1 in A549 cells. *J. Clin. Virol.* **77**, 21–28 (2016).
- 925 102. Knight, Z.A. *et al.* Molecular profiling of activated neurons by phosphorylated
926 ribosome capture. *Cell* **151**, 1126–1137 (2012).
- 927 103. Cong, L. Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**,
928 819–823 (2013).
- 929 104. Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C. & Doudna, J.A. DNA
930 interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature* **507**, 62–67
931 (2014).
- 932 105. Shalem, O., Sanjana, N.E. & Zhang, F. High-throughput functional genomics
933 using CRISPR-Cas9. *Nat Rev Genet* **16**, 299–311 (2015).
- 934 106. Wright, A.V., Nuñez, J.K. & Doudna, J.A. Biology and applications of CRISPR
935 systems: harnessing nature’s toolbox for genome engineering. *Cell* **164**, 29–44
936 (2016).
- 937 107. Ketting, R.F. The many faces of RNAi. *Dev Cell* **20**, 148–161 (2011).
- 938 108. Qi, L.S. *et al.* Repurposing CRISPR as an RNA-guided platform for sequence-
939 specific control of gene expression. *Cell* **152**, 1173–1183 (2013).
- 940 109. Liu, X.S. *et al.* Editing DNA methylation in the mammalian genome. *Cell* **167**,
941 233–247 (2009).
- 942 110. Katz, P.S. ‘Model organisms’ in the light of evolution. *Curr. Biol.* **26**, R649–
943 R650 (2016).

- 944 111. Saltz, J.B. Genetic composition of social groups influences male aggressive
945 behaviour and fitness in natural genotypes of *Drosophila melanogaster*. *Proc. R. Soc.*
946 *Lond. B Biol. Sci.* **280**, 20131926 (2013).
- 947 112. Egan, R.J. *et al.* Understanding behavioral and physiological phenotypes of stress
948 and anxiety in zebrafish. *Behav. Brain Res.* **205**, 38–44 (2009).
- 949 113. Amdam, G.V. & Page Jr., R.E. The developmental genetics and physiology of
950 honeybee societies. *Anim. Behav.* **79**, 973–980 (2010).
- 951 114. Simola, D.F. *et al.* Epigenetic (re)programming of caste-specific behavior in the
952 ant *Camponotus floridanus*. *Science* **351**, aac6633 (2016).

953

954 **Annotated Bibliography**

- 955 5. Rittschof, C.C. & Robinson, G.E. Genomics: moving behavioural ecology beyond the
956 phenotypic gambit. *Anim. Behav.* **92**, 263–270 (2014).

957 Elaborates how the concept of the phenotypic gambit, or the that genetic mechanisms will
958 not inhibit evolutionary trajectories, and genomics can be synthesized to advance
959 behavioral ecology.

960

961

- 962 8. Bell, A.M. & Dochtermann, N.A. Integrating molecular mechanisms into quantitative
963 genetics to understand consistent individual differences in behavior. *Curr. Opin.*
964 *Behav. Sci.* **6**, 111–114 (2015).

965 Aligns core concepts of quantitative genetics to the study of animal personality.

966

- 967 19. Fitzpatrick, M.J. *et al.* Candidate genes for behavioural ecology. *Trends Ecol. Evol.*
968 **20**, 96–104 (2005).

969 Outlines how the candidate gene approach, which utilizes the conserved nature of many
970 genes, can overcome challenges associated with using some molecular tools, such as
971 prohibitively large sample sizes.

972
973 23. Rittschof, C.C. *et al.* Neuromolecular responses to social challenge: Common
974 mechanisms across mouse, stickleback fish, and honey bee. *Proc. Natl. Acad. Sci.*
975 201420369 (2014). doi:10.1073/pnas.1420369111
976 Demonstrates that similar neurological, hormonal and transcriptional changes occur in
977 response to the same social cue across very disparate animal taxa, suggesting conserved
978 genetic 'toolkits' with deep evolutionary origins.
979
980 31. Shaw, K.L. & Lesnick, S.C. Genomic linkage of male song and female acoustic
981 preference QTL underlying a rapid species radiation. *Proc. Natl. Acad. Sci.* **106**,
982 9737–9742 (2009).
983 Investigates the genetic architecture of song preferences across two closely related
984 species of cricket.
985
986 36. Bell, A.M. & Robinson, G.E. Behavior and the dynamic genome. *Science* **332**, 1161–
987 1162 (2011).
988 Proposes that behavioral genomics may lend support to the often-dismissed idea of
989 inherited phenotypic plasticity through genetic assimilation.
990
991 71. Chandrasekaran, S. *et al.* Behavior-specific changes in transcriptional modules lead
992 to distinct and predictable neurogenomic states. *Proc. Natl. Acad. Sci.* **108**, 18020–
993 18025 (2011).
994 Reveals that foraging, maturation and aggression result in different transcriptomic
995 profiles, which in turn create separate neurogenic states.
996
997 86. Gibson, G. & Muse, S.V. *A Primer of Genome Science*. (Sinauer Associates, 2009).
998 A must-read primer on genomics aimed at an audience at the same level as this article.
999

1000 90. Baker, E. J., Jay, J.J., Bubier, J.A., Langston, M.A. & Chesler, E.J. GeneWeaver: a
1001 web-based system for integrative functional genomics. *Nucleic Acids Res.* **40**,
1002 D1067–D1076 (2012).
1003 An introduction to the online gene ontology database GeneWeaver, which is the best
1004 repository currently available for behavior.
1005

		Requires annotated reference genome	Tissue Specific	Developmental stage specific	Cost Per sample*	Requires prior knowledge of target sequence?	Must kill organism?	Pooling or keeping samples separate required?	Estimate of independent samples needed for analyses
	Sequenced and Assembled Genome	N/A	No	No	\$1500+	No	No	Pooling samples necessary	N/A
Genotyping Molecular Markers	RAD-Seq	No	No	No	\$500+	No ***	No	May require pooling if starting tissue is small	10s-1000s+****
	GBS	No	No	No	\$500+	No ***	No	Separate	10s-1000s+****
	Exome capture	Yes	No	No	\$750+	Yes	No	Separate	10s-1000s+****
Comparative Gene Regulation	RNA-Seq	No, but recommended	Yes	Yes	\$500+	No	Yes**	May require pooling if starting tissue is small, but usually inadvisable	10+
	Methylome	No	Yes	Yes	\$1200+	No	Yes**	Separate	3-10+
	ChIP-Seq	Yes	Yes	Yes	\$415+	No	Yes**	Separate	3-10+
	Proteome	No, but recommended	Yes	Yes	\$3500+	No	Yes**	Separate	3-10+
Genetic Manipulations	CRISPR-Cas9 - germline	Yes	No	Must be germline	\$4500+****	Yes	N/A	N/A	N/A
	CRISPR-Cas9 and dCas9-postmitotic	Yes	Yes	No	\$4500+****	Yes	N/A	N/A	N/A
	RNAi	Yes	Sometimes	-	\$50+	Yes	N/A	N/A	N/A

* Price per samples estimate for a whole project, including analysis, based on storefront costs of the Technology Center for Genomics and Bioinformatics at UCLA. URL:<<https://www.scienceexchange.com/labs/technology-center-for-genomics-bioinformatics-ucla>>, with the exceptions of Proteome (from MS Bioworks; URL: <<http://www.msbioworks.com/services/protein-works/protein-profiling>>), CRISPR-Cas9 preps (Transgenic & Gene Targeting Core, University of Utah; URL: <<https://www.scienceexchange.com/labs/transgenic-gene-targeting-core-utah>>), and RNAi (ThermoFisher Scientific; URL: <<http://www.thermofisher.com/us/en/home/life-science/rnai/vector-based-rnai/pol-ii-mir-rnai-vectors.html>>)

** These methods require the isolation of specific tissue at specific life stages. For behavioral studies, isolation of the brains will usually be preferable.

*** Prior knowledge of genome allows estimation of density of restriction sites present throughout the target genome, and thus allows a more informed selection of restriction endonuclease based on volume of restriction sites in the target genome.

**** Sample sizes depend on intended use. Population genomics and SNP screening will require a few samples per groups, while association studies (GWAS, QTL mapping) require massive sample sizes to attain good statistical power. In some cases, association studied may be done with <100 individuals given stringent conditions (see text)

***** Price per preparation for transgenic model organism preparation

	Massively Parallel Sequencing				Comparative Gene Regulation				Experimental methods		
	Sequenced and Assembled Genome	RAD-seq/ Genotyping-by-Sequencing	Candidate gene region	Exome capture	RNA-Seq	Methylome	ChIP-seq	Proteome	CRISPR	CRISPR	RNAi
									germ-line	postmitotic	
Why do individuals behave consistently?	Consistent interindividual differences in behavior result from major detectable changes in the genomes.	Consistent interindividual differences in behaviors result directly from interindividual sequence and/or structure differences in association with genomic markers	Consistent interindividual differences in behaviors result directly from interindividual sequence differences in a given candidate genetic region	Consistent interindividual differences in behaviors result directly from interindividual differences in protein sequences.	Consistent behaviors are the result of genetic networks being expressed in the same circumstances.	Comparing the methylome of individuals among behavioral types can explain the degree of consistency within/between behavioral types.	Consistent behaviors result from epigenetic constraints preventing the expression of alternative phenotypes	See RNA-Seq	Affecting the genome sequence of loci identified as causing consistent behaviors affects the behaviors as well.	Modulating the expression of genes involved in the expression of consistent behaviors affects the behavior.	Modulating the expression of genes involved in the expression of consistent behaviors affects the behavior.
Why are traits correlated?	X	Correlated behavioral traits are expressed by pleiotropic or closely linked genes.	See RAD-seq/GBS, testable with more precision. This requires the prior knowledge of candidate regions	Correlated traits are expressed via pleiotropic changes	Correlated traits are governed by genes involved in the same transcription regulatory network, enhanced by the same TFs	Correlated traits are governed by genes correlated in their methylation states	Genes coding for correlated traits become accessible through epigenetic changes involving the same TFs and/or histones	Expression of correlated traits involve multiple protein variants folded from the same amino acid chains through post-translational modifications	Correlated traits are affected by pleiotropic genes. Thus, affecting the function of genes involved changes the expression of all correlated behaviors	Correlated traits are affected by the expression of pleiotropic genes in the different tissues and/or at different life stages	Correlated traits are affected by the expression of pleiotropic genes in the different tissues and/or at different life stages
Why are there differences in developmental plasticity?	X	Differences in the level of plasticity for a trait result from differences in the sequence and/or structure of the genome.	Difference in the level of plasticity in behavior result directly from sequence differences in a given candidate genetic region	Differences in the level of plasticity in behaviors result directly from differences in protein sequences of each morph	Differences in the level of plasticity in behavior results from differences visible through transcriptomes (e.g. changes in the transcript regulatory networks, changes in TF binding sites)	Differences in the level of plasticity in behavior scales with differences in levels of DNA methylation between plastic and non-plastic morphs following critical developmental period	Differences in the level of plasticity in behavior result from epigenetic differences at the critical developmental time, rendering genomic regions involved in plasticity inaccessible	Differences in the level of plasticity in behavior results from differences in maternal-effect proteins available at critical times (may also visible through transcripts)	Modifying genetic regions involved in the plastic response to an environment will significantly alter the plasticity of a trait.	Preventing the transcription of genes with modular expression across a reaction norm will render a plastic behavioral traits canalized.	Blocking the translation of maternal-effect mRNA identified as being involved in differences in plasticity will canalize the expression of a trait.
Why are there differences in contextual plasticity?	X	Differences in the level of plasticity for a trait result from differences in the sequence and/or structure of the genome.	Difference in the level of plasticity in behavior result directly from sequence differences in a given candidate genetic region	Differences in the level of plasticity in behaviors result directly from differences in protein sequences of each morph	Differences in the level of plasticity in behavior results from differences visible through transcriptomes (e.g. changes in the transcript regulatory networks, changes in TF binding sites)	Differences in the level of plasticity in behavior scales with differences in levels of variance in DNA methylation following a cue.	Differences in the level of plasticity in behavior result from differences in the scale and shape of epigenetic modifications between morphs.	See RNA-Seq	Modifying genetic regions involved in the plastic response to an environment will significantly alter the plasticity of a trait.	Preventing the transcription of genes with modular expression across a reaction norm will render a plastic behavioral traits canalized.	Knocking down the transcription of genes with modular expression across a reaction norm will render a plastic behavioral traits canalized.
Why are there differences in behavioral type?	There are major genomic differences between types	Behavioral types are the result of genetic changes in sequence and/or structure in the genome.	Behavioral types are the result of sequence change in specific candidate genetic regions. Detectable with more power than in genome-wide methods	Behavioral types are the result of sequence change in the protein-coding sequences between morphs	Behavioral types are the result of major changes in transcription regulatory networks between types.	Behavioral types are the result of profound and maintained differences in methylation, especially around genes involved in the expression of divergent behaviors	Behavioral types are the result of profound and maintained epigenetic differences between types	Differences in post-translational processes contribute to differences in behavioral types.	Genetic modifications of divergent sequences from it's state in one type to its state in another type will result in a reduction of the phenotypic differences between types	Modulating the gene expression of genes with differences in expression between types, especially in the brain, will result in changes in the phenotypes of behavioral types	Modulating the gene expression of genes with differences in expression between types, especially in the brain, will result in changes in the phenotypes of behavioral types

Why are there differences in behavioral plasticity?

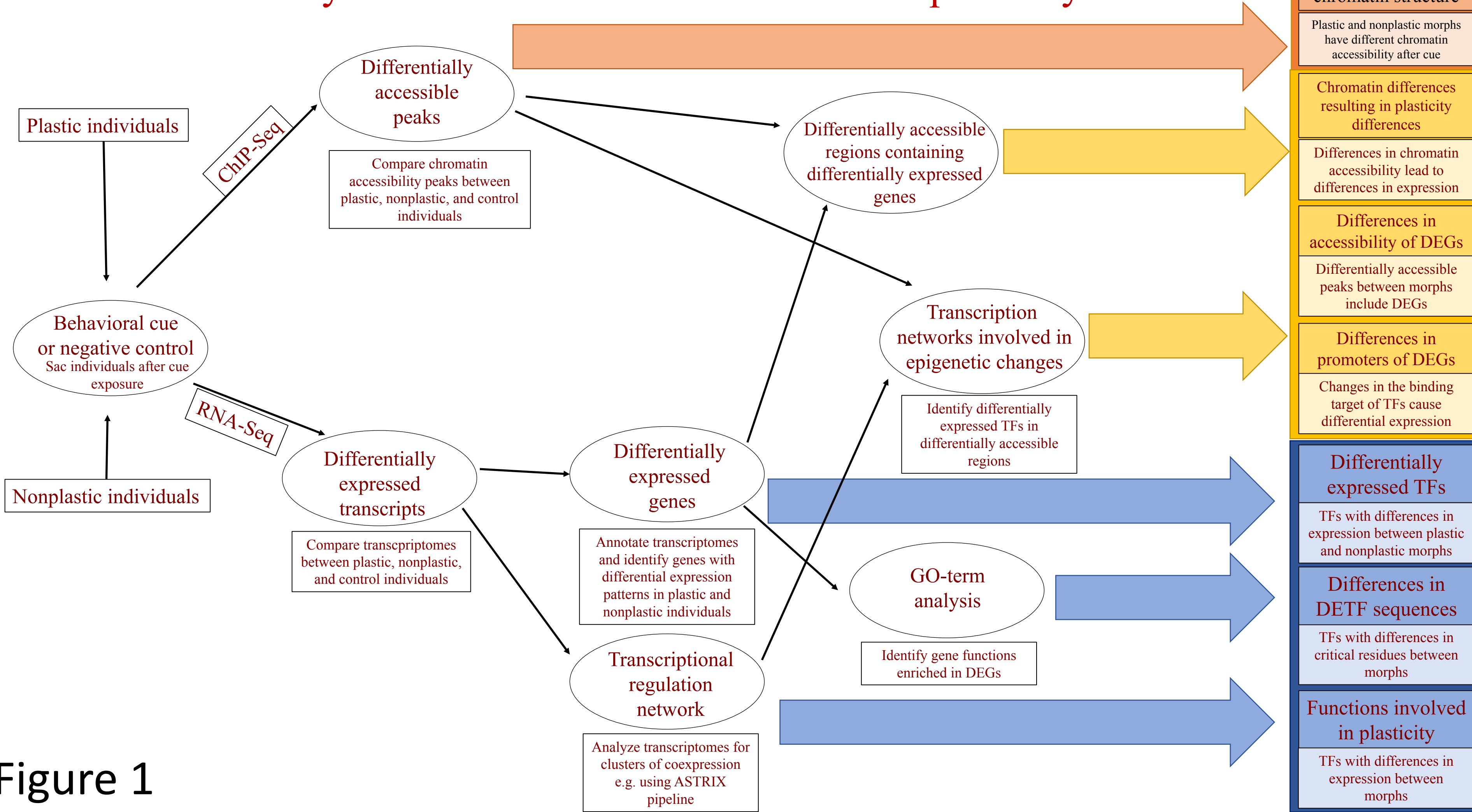


Figure 1