RESEARCH PROGRAM
My research program is encapsulated by the six major projects described below, with supporting publications where relevant.

1. MHC controlled susceptibility to infectious disease. We were the first to show experimentally that MHC heterozygote advantage seldom occurs for a given infectious agent, but when two infectious agents with opposite MHC susceptibility-resistance profiles infect a host, then heterozygote advantage emerges because resistance tends to be dominant, or partially dominant, over susceptibility. Recently, we have shown that MHC variation sculpts microbiome communities. Furthermore, transplanting the microbiome from one MHC-congenic mouse strain into germ-free individuals of another MHC congenic strain causes the recipient to express the susceptibility profile of the donor strain. This suggests a relatively novel mechanism for the pervasive associations between MHC and susceptibility to infectious disease.

a. 2002 Penn, D.J., K. Damjanovich, W.K. **Potts**. MHC-heterozygosity confers a selective advantage against multiple-strain infections. *Proc. Nat. Acad. Sci*. 99:11260-11264. PMCID: PMC123244
b. 2003 McClelland, E.E., D.J. Penn, W.K. **Potts**. Major Histocompatibility Complex heterozygote superiority during coinfection. *Infection and Immunity* 71:2079–2086. PMCID: PMC152037
c. 2007 Ilmonen, P. D.J. Penn, K. Damjanovich, L. Morrison, L. Ghotbi and W.K. **Potts**. Major histocompatibility complex heterozygosity reduces fitness in experimentally infected mice. *Genetics* 176:2501-2508. PMCID: PMC1950649
d. 2015 Kubinak, J.L., W. Z. Stephens, R. Soto, C. Petersen, T. Chiaro, L.G., R. Bell, N.J. Ajami, J.F. Petrosino, L. Morrison, W.K. **Potts**, P.E. Jensen, R.M. O'Connell1 & J.L. Round. MHC variation sculpts individualized microbial communities that control enteric infection. *Nature Communications*. 6:8642. doi: 10.1038/ncomms9642. PMID:26494419; PMCID: PMC4621775

2. Genes of the major histocompatibility complex (MHC) function in mate choice and kin recognition. In our initial attempt to discover the evolutionary forces maintaining the unprecedented genetic diversity observed for genes of the major histocompatibility complex, we measured the selective forces operating on MHC genes in large semi-natural populations of house mice. We expected to find either heterozygote advantage or evidence for antagonistic coevolution (a molecular arms race between pathogens and hosts, also known as the red queen hypothesis). In each of nine independent populations we observed a deficiency of MHC heterozygous offspring, which was a prediction of the heterozygote advantage hypothesis. However, we were able to show that the heterozygote deficiency was actually due to females preferring to mate with MHC dissimilar males. The immunological community was skeptical of this result for many years, but similar mating preferences have now been shown in over 20 species and a mechanism has now been demonstrated. It appears that MHC mating preferences will be a general vertebrate trait that functions to produce offspring with more immuno-competent genotypes or to avoid inbreeding. In support of the inbreeding avoidance hypothesis, we have shown that MHC functions in kin recognition during the choice of communal nesting and nursing partners in females.

a. 1991 **Potts**, W.K., Manning, C.J., Wakeland, E.K. Mating patterns in semi-natural populations of mice influenced by MHC genotype. *Nature* 352:619-621. PMID: 1865924
b. 1992 Manning, C.J., Wakeland, E.K., **Potts**, W.K. Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature* 360:581-583. PMID: 1461279
c. 1993 **Potts**, W.K., E.K. Wakeland. The evolution of MHC genetic diversity: a tale of incest, pestilence, and sexual preference. *Trends in Genetics* 9:408-412. PMID: 8122307
d. 2002 Carroll, L.S., D. Penn, W.K. **Potts**. Discrimination of MHC derived odors by untrained mice is consistent with divergence in peptide-binding region residues. *Proc. Nat. Acad. Sci.* 99:2187-2192. PMCID: PMC122340

3. Pathogens rapidly adapt to host genotypes, but with a tradeoff cost for infecting other genotypes. We have used experimental evolution techniques to test hypotheses about host-pathogen coevolution. Pathogens adapt to host genotypes, including MHC genotypes alone and these adaptations to one genotype carry tradeoff costs in the pathogen's ability to adapt to other host genotypes. This is an essential element of host-pathogen antagonistic coevolution (molecular arms races), which had been long predicted, but these studies were the first to empirically demonstrate this for a vertebrate. We were also the first to experimentally test and confirm a corollary hypothesis that host genetic diversity impedes pathogen virulence evolution. This fundamental discovery also has implications for captive breeding of endangered species and the problem of overuse of antibiotics in domestic animals.

a. 2012 Kubinak, J.L., J.S. Ruff, C.W. Hyzer, P.R. Slev, and W.K. **Potts.** Experimental viral evolution to specific host MHC genotypes reveals fitness and virulence tradeoffs in alternative MHC types. *Proceedings of the National Academy of Sciences USA*. 28;109(9):3422-7. PMCID: PMC3295311 (Cover photo article, 28 Feb. issue)
b. 2013 Kubinak, J.L., J.S. Ruff, C.W. Hyzer, P.R. Slev, W.K. **Potts.** MHC versus non-MHC as the primary target of pathogen adaptation. *Genes and immunity*. 14**:**365–372. PMCID: PMC4517933
c. 2015 Kubinak, J.L., Douglas H. Cornwall, Frederick R. Adler, Kim J. Hasenkrug and W.K. **Potts.** Serial infection of diverse host (Mus) genotypes rapidly impedes pathogen fitness and virulence. *Proceedings of the Royal Society of London B.* 282:1798:20141568. PMCID: PMC4262163
d. 2021 Middlebrook EA, Stark DL, Cornwall DH, Kubinak JL, **Potts** WK (2021) Deep sequencing of MHC-adapted viral lines reveals complex recombinational exchanges with endogenous retroviruses leading to high-frequency variants. *Frontiers in Genetics*. 12:716623. doi: 10.3389/fgene.2021.716623

4. Organismal Performance Assays (OPAs) provide sensitive assessment of health degradation from genetic and other treatments. Our current toxicity assessment methodologies are inadequate. Consequently, we expose the public to substances whose toxicity is only discovered later, usually after decades of epidemiology. We are in effect, experimenting on ourselves and it is likely that toxicants (including diets with toxic effects [e.g., those high in refined sugar] that lead to non-communicable diseases are the greatest source of health degradation in humans (U. N. General Assembly, 2012). In response we have been developing a technique to assess toxicity that we originally developed for quantifying adversity from genetic manipulations. Our assay uses wild mice in semi-natural enclosures where treated and control mice compete directly for territories, resources, and mates. Our model achieves unparalleled sensitivity and breadth because high performance from most physiological

systems is required for reproductive and social success in this competitive environment. Consequently, any exposure that reduces performance of any physiological system (e.g., cardiorespiratory, metabolic, etc.) will likely be detected. We call this methodology an Organismal Performance Assay (OPA) and it has proven 50 times more sensitive than previous methods in detecting the adverse effects of inbreeding (Meagher et al., 2000) and was the first assay that discovered toxicity of dietary added sugar at human-relevant levels (Ruff et al., 2013). Furthermore, for two pharmaceuticals that each had passed approximately 1 billion dollars of safety testing, but when released for use by millions of Americans, unacceptable side effects were encountered, resulting in one being recalled (cerivastatin) (Gaukler et al., 2016) and the other receiving a black box warning (Paxil)(Gaukler et al., 2015). We detected health declines for both substances at a cost that was four orders of magnitude less than the billion dollars of safety testing they passed. We have also demonstrated health problems from the replacement of one Hox gene with a Hox paralog; the original study published in *Nature* reported that these mice were phenotypically normal. Combined, these results suggest that OPAs have the ability to detect toxicities missed by conventional methodologies and will be an important addition to health and safety testing for new pharmaceuticals, genetic manipulations and environmental exposures.

a. 2000 Meagher S., D.J. Penn, and W.K. **Potts**, Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Nat. Acad. Sci. 97:3324-3329.* PMCID: PMC16238
b. 2015 Gaukler, S.M., J.S. Ruff, T. Galland, K.A. Kandaris, T.K. Underwood, N.M. Liu, E.L. Young, L.C. Morrison, G.S. Yost, W.K. **Potts**. Low-dose paroxetine exposure causes lifetime declines in male mouse body weight, reproduction and competitive ability as measured by the novel organismal performance assay. *Neurotoxicology and teratology*. 47:46-53. PMCID: PMC4416947
c. 2013 Ruff, J.S., Suchy, A.K., Hugentobler, S.A., Sosa, M.M., Schwartz, B.L., Morrison, L.C., Gieng, S.H., Shigenaga, M.K. & **Potts**, W.K. Human-relevant levels of added sugar consumption increase female mortality and lower male fitness in mice. *Nat Commun. 4:2245*. PMCID: PMC3775329
d. 2015 Ruff, J.S., Saffarini, R.B., Ramoz, L.L., Morrison, L.C., Baker, S., Laverty, S.M., Tvrdik, P. & **Potts**, W.K. Fitness assays reveal incomplete functional redundancy of the HoxA1 and HoxB1 paralogs of mice. *Genet. 201: 727-736.* PMCID: PMC4596679

5. Epigenetic transgenerational inheritance of high-cost social pheromones. Parents exposed to our semi-natural populations produce sons that realize 40% higher reproductive success than sons from parents breeding in conventional housing; this is despite the fact that neither type of offspring had experienced semi-natural conditions prior to founding and competing under semi-natural conditions. We have shown that offspring with semi-natural ancestry produce higher levels of MUPs (major urinary proteins), which are excreted in mg/ml quantities of urine. These high cost MUPs act as social signals in territory formation and mate attraction. We demonstrated that this MUP upregulation is associated with demethylation of a MUP promoter region. We also demonstrated that expression levels of a particular MUP predict winners of social dominance (as does body mass and locomotor economy).

a. 2013 Nelson, A.C., K.E. Colson, S. Harmon, W.K. **Potts.** The role of sexual selection during rapid adaptation to a socially competitive environment. *BMC Evolutionary Biology*. 13:81-95. doi: 10.1186/1471-2148-13-81.
b. 2013 Nelson, A.C., J. W. Cauceglia, S.D. Merkley, N.A. Youngson, A.J. Oler, R.J. Nelson, B.R. Cairns, E. Whitelaw, W.K. **Potts.** Reintroducing domesticated wild mice to

sociality induces adaptive transgenerational effects on MUP expression. *Proceedings of the National Academy of Sciences USA*. 110:19848–19853. PMCID: PMC3856842

c. 2015 Nelson, A.C, C. Cunningham, J.S. Ruff, W.K. **Potts**. Protein pheromone expression levels predict and respond to the formation of social dominance networks. *Journal of Evolutionary Biology*. 28: 1213–1224. PMCID: PMC4478133

d. 2017 Ruff, J.S., Cornwall, D.H, Morrison, L.C., Cauceglia, J.W., Nelson, A.C., Gaukler, S.M, Meagher, S.M., Carroll, L.S., and **Potts**, W.K. Sexual selection constrains the body mass of male but not female mice *Ecol Evol*. 7: 1271-1275. PMCID: PMC5306010 (Cover photo article, Feb. issue)

6. Transmission of malaria from asymptomatic individuals in mice and men

Malaria kills 600,000 individuals per year, most of which are children under five years of age. Malarial disease is caused by transmission of *Plasmodium* parasites and transmitted to humans through the bite of infected *Anopheles* mosquitoes. Transmission by asymptomatic carriers plays a major role in the dynamics of many pathogens ranging from SARS-CoV-2 to malaria. The majority of the population in many malaria endemic areas (>60%) are asymptomatic (without overt symptoms), even in high transmission areas. Progress has been hindered by the lack of an animal model. We have recently developed the first animal model that allows us to experimentally test a myriad of hypothesis associated with asymptomatic malaria. In collaboration with Tracey Lamb (Department of Pathology), we currently have two major federal grant proposals under review aimed at tackling these problems (see above).