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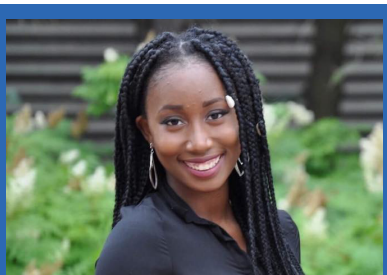

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Ethics and the Neurological Effects of Water Contamination by Methylmercury

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Abstract

What ethics-related discussion is present in journal articles on brain damage due to water contamination by methylmercury? As a neurotoxin, increased bioavailability of methylmercury driven by human activities can have a significant adverse impact on future generations. We categorized ethics-related content according to a framework for Environmental Neuroethics (Cabrera et al., 2016). The framework provides a systematic way of examining phenomena at the intersection of ethics, brain, and environmental change. Measures of the relative quantity of ethics-related content and sources of academic discourse were also made. The most extensive ethical discussion concerned implications for social policy and regulation. We also noted a lack of ethics-related content with regard to cross-cultural perspectives.

Introduction

Methylmercury (MeHg) is a potent neurotoxin, especially for a fetus exposed in utero. Research into its effects in brain health evolved after the fall-out of major anthropogenic mercury pollution incidents in Japan (1956, 1965) and Iraq (1971) (Harada, 1995; Amin-Zaki et al., 1974). MeHg is mercury (Hg) in its fat soluble organic form, which readily passes through the blood brain barrier (Järup, 2003). Brain damage resulting from exposure in adulthood is focal, and primarily causes impairment of movement, vision and audition as well as cognitive impairments (Castoldi et al., 2001). Prenatal exposure impairs normal neurodevelopment and leads to widespread neuronal degradation. Expressions of such MeHg-induced neurotoxicity can take a variety of forms, including cerebral palsy and autism spectrum disorder.

MeHg is produced through methylation of inorganic Hg by aquatic micro-organisms (Clarkson, 1995). Increasing anthropogenic emissions of mercury through industrial practices have increased the environmental availability of Hg as well as rates of conversion to its neurotoxic form MeHg (Booth and Zeller, 2005). This conversion of Hg to MeHg poses the greatest threat to brain health, because MeHg bio-accumulates through the food chain (Wang et al. 2004). This may render local waters safe to drink, while fish and marine mammals contain toxic concentrations. The size and age of the fish also contributes to its relative MeHg concentration. For example, king mackerel is significantly more toxic than smaller species of mackerel (Silbernagel et al., 2011). Increased environmental availability of MeHg and subsequent low dose exposure by human populations is implicated in what has been considered as the silent pandemic of neurodevelopmental disorders arising from subclinical neurotoxicity (Labie, 2007; Grandjean and Landrigan, 2006).

Contradictory findings from two major longitudinal studies on the neurological effects of MeHg have sparked debate concerning policy regulation for restricting the consumption of certain foods. One such ongoing study is based in a cohort of children in the Republic of Seychelles, whose pre- and postnatal MeHg exposure results primarily from consumption of ocean fish. This cohort has almost no reported adverse effects on neurodevelopment with increased MeHg exposure; and even show slight improvements on tests of cognitive ability (Davidson et al., 2011). These findings are commonly attributed to the paired increased intake of nutrients beneficial for neurological function that occur naturally in the same fish. In stark contrast, a cohort of children on the Faroe Islands exposed to MeHg primarily through consumption of both fish and whale products have reported significant and lasting cognitive deficits (Debes et al., 2016).

A major motivator for the present study was the Calder et al.'s (2016) report that 11 of 22 planned hydroelectric facilities in Canada will increase MeHg

concentrations in surrounding areas sufficiently to produce an adverse effect in local communities. Many of these are First Nations settlements. Even active efforts to reduce anthropogenic effects on the natural environment lack consideration of potential adverse effects on brain health. No review of the extent or themes present in the ethical discussion has been carried out with regard to methylmercury. To address this knowledge gap, the framework for Environmental Neuroethics (Cabrera et al., 2016) (Fig. 1) was used to investigate the ethical considerations reflected in the academic literature on brain damage due to environmental water contamination by methylmercury. Such an investigation has the potential to address directions for further academic discourse and impact policy decisions.

Framework Environmental Neuroethics	
Brain science and the environment	Neuroscience discovery that is aligned with the measurement and evaluation of factors that affect the way individuals, communities and society adapt and cope with real or perceived environmental threats to well-being.
The relational self and the environment	The interface between the environment and brain, and the mechanisms by which exposures at key points in life may mediate different brain and mental effects; relationships with different environmental stressors, individual susceptibility and resilience.
Cross-cultural factors and the environment	Exploration of the role of culture in the relationship between environment and brain and mental health; interactions between Traditional Ecological Knowledge and neuroscience evidence.
Social policy and the environment	Priorities and allocation of resources of local social organizations to deal with environmental impacts on brain and mental health.
Public discourse and the environment	The engagement of professional disciplines and communities in multidirectional communication and discourse about neurological, psychological, sociological and ethical dimensions of environmental change; facilitation of international, cross-disciplinary, transdisciplinary collaborations.

Figure 1. Framework for Environmental Neuroethics (Cabrera et al., 2016). Reprinted with permission.

Methods

MeSH search terms and PubMed search

As a first step in this scoping review, the research question was formulated into a PubMed search query based on Medical Subject Headings (MeSH). A MeSH search includes both articles indexed according to this system, and other papers under the same keywords in the larger PubMed database. Prior to finalizing the query, different search terms were tested to ensure that the result reflected the research question.

Search terms were extracted from three categories; terms relating to environmental sources of pollution in water environments, terms relating to brain health, and terms relating to methylmercury. The MeSH terms used under the umbrella of environment were: 'environment', 'industry', 'water', 'chemical water pollution' and 'environmental pollution'. Terms related to brain health were; 'neurology', 'neurotoxins', 'chronic brain damage' and 'neurodevelopmental disorders'. The only relevant search term for the compound name was 'methylmercury compounds' (Fig. 2). Terms under an umbrella category were joined by disjunction, and these content categories were then joined by conjunction. This enforced that some subset of terms from each of the three umbrella categories had to appear as search terms for every query return.

ENVIRONMENT	BRAIN	COMPOUND
Environment	Neurology	Methylmercury Compounds
Industry	Neurotoxins	
Water	Chronic Brain Damage	
Chemical Water Pollution	Neurodevelopmental Disorders	
Environmental Pollution		

Figure 2: MeSH search terms for the PubMed query. Column terms were joined by disjunction; and rows by conjunction such that at least one element from each column category must have been a search term used to index a given article.

The search was conducted in March 2017. All returns were manually curated and reviewed by the first author. Articles were excluded if they did not concern impacts on human health (e.g., focused on another species), or were written in a language other than English or French. There was a sharp decline in the indexed coverage of the debate published prior to 1995, so articles prior to January 1995 were also excluded. An additional five articles were excluded because they could not be accessed.

Framework Operationalization

In order to categorize articles consistently according to the framework, each category was operationalized as follows:

- *Brain science and the Environment (BSE):* Discussion relating to measurements of neurotoxicity, prevention methods, physical and/or psychological methodology for detecting, evaluating, preventing, and/or treating neurotoxic effects.

- *Relational Self and the Environment (RSE):* Discussion concerning mental health and the vulnerability to MeHg neurotoxicity at different life stages, comparing pre-/peri- and postnatal effect sizes to that of adult populations, as well as the long-term effects of exposure during neurodevelopment.

- *Cross-Cultural Factors and the Environment (CCFE):* Discussion on how exposure to MeHg differ in populations resulting from a community's cultural practices, such as traditional sources of food and ways of living, as well as differing approaches to neurotoxicity and the knowledge thereof. An article containing mention of differential exposure due to location rather than culture would not fall under this category.

- *Public Discourse and the Environment (PDE):* Discussion of the spread of knowledge and communication concerning neurotoxicity and environmental sources of methylmercury, as well as the effectiveness and implementation of intervention methods. Strongly ties in with education.

- *Social Policy and the Environment (SPE):* Discussion of regulation, legislation and policy-making, and utility calculations that often are based in economic considerations.

Additional Categorizations

In addition to categorizations according to the framework, the extent of ethical discussion was recorded in three broad categories; none, minimal/some, and extensive. An article would fall under 'extensive' if the main purpose of the paper was related to ethics-based discussion; under 'minimal/some' if some content was related to ethics but not the main aim of the paper. While many articles contained ethics-related words such as vulnerability, a quantitative measure of the number of such words did not necessarily correspond to the extent of the ethical discussion. An article would fall under 'none', therefore, if no ethics-related implications were discussed, or an ethics-related content word (such as vulnerable) appeared without more elaborate discussion.

The type of literary source was also recorded; primary research, reviews and other types of sources such as letters were represented in the sample.

Controls

As a measure of consistency, 20% randomly chosen articles were double-coded by an independent researcher in the same lab. The reviewer was supplied with categories and corresponding operationalizations, exclusion criteria, and the code for evaluating the extent of the ethical discussion. After a first pass, the results and implications on the broader search were discussed, and categorizations were re-evaluated until consensus was reached. The remaining articles were subject to a second pass after this review.

Results

The search returned 108 papers, of which 91 met inclusion criteria. Among the 91 articles, 44 (48%) had no ethics-related content, 27 (30%) had minimal/some discussion, and 20 (22%) contained extensive ethical discussion (Fig. 3). Among the 47 articles with ethics-related content, 19 (40.5%) originated in primary research and 25 (53%) in reviews. Three articles (6.5%) were letters and elaborations on previous research.

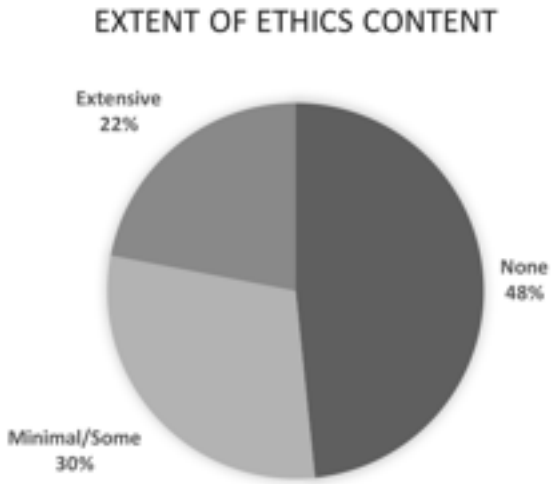

Figure 3. Proportion of articles containing extensive, minimal/some, and no ethical discussion (N=91).

Among articles with ethics-related discussion, 15 were categorized under BSE, 6 under RSE, 2 under CCFE, 8 under PDE and 16 under SPE (Fig. 4). Categorizations reflect the primary theme in the article's ethics-related content. Articles with no ethical content were not categorized.

The extent of ethics-related discourse in the analyzed papers varied significantly across framework

categories. The highest proportion of extensive discussion (10 extensive, 6 minimal/some) was found in SPE, while the highest proportion of minimal/some ethical discussion (12 minimal/some, 3 extensive) was found in BSE. Both PDE and CCFE had an equal split, while RSE had extensive ethical discussion in 2 among its total of 6 papers.

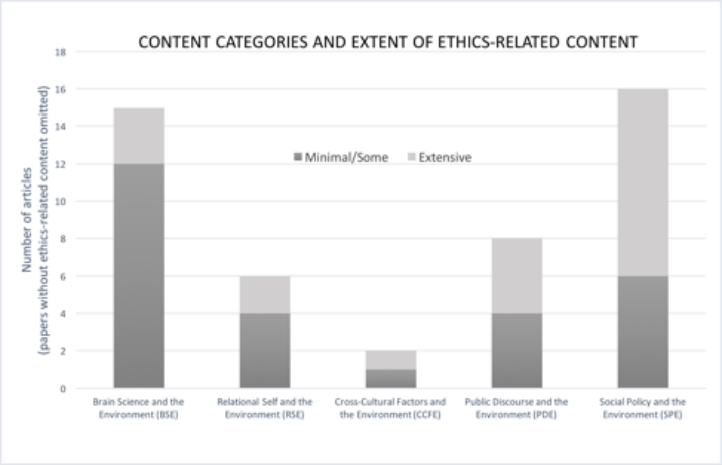

Figure 4. Number of articles among those with ethics-related content separated according to framework category and extent of discussion.

In terms of category distribution between types of literature, there was a roughly equal split between primary research and reviews overall. The most significant difference was under PDE, were a majority of articles were reviews. The distribution of primary among the total number of articles (primary / total), were; BSE: 7/15, RSE: 3/6, CCFE: 1/2, PDE: 2/8 (one letter included in total), and SPE: 6/16 total (two letters included in total) (Fig. 5). Among review articles containing some ethics-based discourse, 14 out of 25 discussed the topic extensively. In papers originating from the primary literature, this proportion shifted dramatically to 3 out of the total 19. Two out of the three letters and elaborations to previous research contained extensive ethics-based discussion.

Discussion

The categorizations offered by the framework for Environmental Neuroethics provided insight into ethics-related contributions to the academic discussion about brain and methylmercury (MeHg). The category Social Policy and the Environment (SPE) contained the greatest proportion of articles with any ethics content overall. These articles were also the most likely to contain extensive ethical discussion. SPE and Public Discourse and the Environment (PDE) included the greatest proportion of review articles relative to primary research articles. Since methylmercury has long been known for its neurotoxic effects, the results seen for SPE and PDE reflect the contemporary debate concerning evaluation of reference dosages, suggestions for health and safety policies, and the impact on society from education and implementation of regulatory practices.

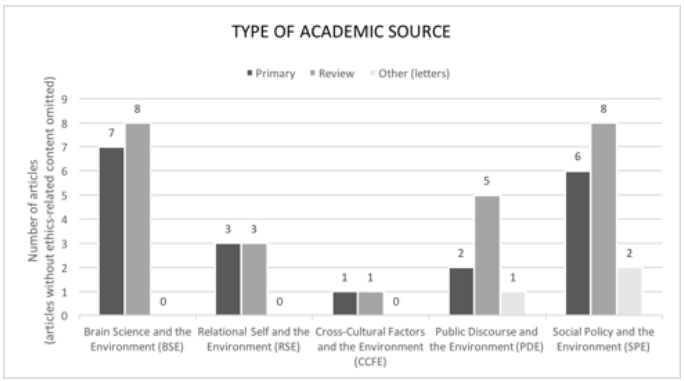

Figure 5. Number of articles among those with ethics-related content according to category and type of literary source.

The longitudinal studies in the Republic of Seychelles and Faroe Islands highlight the importance in making policy decisions and public advisories that both reduce the risk of MeHg neurotoxicity and maintain intake of beneficial nutrients from seafood. This is an especially important issue among vulnerable populations such as women of childbearing age and young children, as well as within indigenous communities where alternative sources of food may be neither readily accessible nor desired. Attempts to achieve a balanced advisory for fish consumption are reflected in articles categorized under SPE. These articles contained evidence of economic estimates as a proxy for neurological damage, through for example a drop in IQ, resulting from adherence to different regulatory practices. (See for example Bartlett and Trasande, 2014). The focus in Relational Self and the Environment (RSE) on consequences of exposure during different stages of neurodevelopment suggest a strong link to RSE and how regulation influence these effects.

It is perhaps not surprising that the greatest proportion of primary studies as well as articles cont-

aining only minimal/some ethics-related content were reported in the category Brain Science and Environment (BSE). Even though ethical considerations fell outside the primary focus of these papers, they were often present to warrant the relevance of presented research, or to stress the importance of findings. Many articles in this category focused on the neurological basis for neurotoxicity due to methylmercury exposure, as well as determinations of the effect size of confounding variables in epidemiological studies.

Given the characteristics of the neurotoxic effects from methylmercury exposure, it was not uncommon for articles to contain ethics-related discussion suitable for coding into multiple categories. In such cases, the ethics-related content was categorized according to the primary theme in the discussion. This may however have contributed to what appears to be a restricted discussion in the categories Relational Self (RSE), Public Discourse (PDE) and Crosscultural Factors (CCFE). An example of such a paper is "Maternal Fish Consumption and Prenatal Methylmercury Exposure: A Review" (Al-Ardhi and Al-Ani, 2008). This paper was categorized under BSE, owing to a focus on how properties of fish and consumption patterns contribute to producing neurotoxic effects of MeHg. However, the same paper also uses its findings to extensively engage in discussion concerning policy and regulation for public advisories, especially with regards to the unique vulnerability of the child in-utero. The relevance to themes in PDE, SPE and RSE ought also to be reflected in the classification of such articles. For future studies using the same framework, adding the dimension of relative content between categories would enrich the findings and possible conclusions.

Categorizing articles according to the framework permitted an insight into where contribution to the debate is lacking. In this study, this was most evident with regards to crosscultural perspectives. Only two out of 47 papers contained ethical discussion focused on themes under CCFE: both case studies of populations highly exposed to methylmercury through traditional ways of living. Communities that rely predominantly on wild caught fish as a source of dietary protein are especially vulnerable to methylmercury exposure. Such communities, like the Canadian First Nations, often differ in their cultural practices compared to the country's mainstream culture, and live in areas with high risk of undergoing changes due to industrial expansion. These topics are present in the academic literature, as exemplified by the debate surrounding Canadian prospective hydroelectric power plants, but are underrepresented in this search.

PubMed provides access to the extensive MEDLINE database of biomedical research. We carefully curated returns to provide precise search results. PubMed is, however, limited papers published in life science journals. In the future, extended analyses that include papers from other search engines such as Google Scholar will provide further foundational content for related academic and regulatory pursuits in environmental neuroethics.

Acknowledgements

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Exploring Systems of Reprodution through Modification of a Genetic Algorithm aimed at Optimizing Molecular Geometry

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Abstract

The main objective of this study was to compare the efficiency of various evolutionary systems in maximizing the fitness of the population upon which they act. This was accomplished through the development of a genetic algorithm to optimize the chemical structure of carbon dioxide (CO2), which has a precisely known solution. This allowed for the comparison of efficacy of different versions of the algorithm, each based on a different evolutionary strategy. Each breeding system represents a distinct approach to reproduction, generating unique evolutionary curves; however, the population fitnesses converge toward the optimized solution at approximately the same time. It is concluded that for the given problem all versions are practical, but for more complex problems the path taken to optimization might make one method preferable.

Introduction

Genetic algorithms (GAs) provide a means of solving complex optimization problems. The process is analogous to natural selection acting on a population to induce evolution, including concepts such as fitness, mutation, and crossover (Mitchell, 1998). GAs are used across industries to solve optimization problems in everything from shipping routes and gene expression analysis to investment strategies and chemical reaction pathways (Ross & Corne, 1994). When the algorithm is first initiated, a population of candidate solutions is randomly generated, each with their own genome, which contains all parameters necessary to describe the phenotype. These individuals are then reproduced in an iterative process, producing generations of solutions, with each individual assigned a fitness score. The score is indicative of how close an individual is to the ultimate solution relative to other candidates, and depends on what problem the population is meant to solve (Whitley, 1994). Mutations in the genome may also occur, controlled by a fixed mutation rate. This allows successive generations to score progressively higher in the fitness module, and thus evolve the population towards maximum fitness. The fundamental premise of genetic algorithms is that biological evolution provides a highly effective mode of optimization. This paper aims to extend this analysis beyond random mating, introducing explicit systems of reproduction in order to quantitatively investigate their efficiency outside of their intended biological context.

In this work, a genetic algorithm was written with the aim of optimizing molecular geometry by minimizing the energy of interaction between each of the molecule's constituents. CO2 was used as an exemplar, as it can be modeled with a relatively low number of parameters, which translates into a genome of a manageable size. This project could have been conducted on any optimization problem, but this one was chosen because its precise solution is known. Similar molecular geometry optimization problems have been solved with genetic algorithms, particularly those involving organic molecules. One notable example comes from a 1995 paper by Deaven & Ho, wherein the structure of fullerenes is predicted from an initial set of random coordinates, up to the buckyball sphere structure of C_{60}.

Our experiment investigates the evolutionary role of various reproductive strategies observed in the natural world, as well as how they affect the fitness of the populations upon which they act. We aim to provide a quantitative foundation from which to approach these biological questions through the disciplines of

chemistry, mathematics, physics, and computer programming.

Methods

Program Development

Figure 1 illustrates the structure of the genetic algorithm. Because many generations are often needed to find an optimal solution, maximizing efficiency in genetic algorithms is key. We found that the most efficient way to encode information in our program was to represent the population of molecules in a 2D array, each row of which contains sufficient parameters to describe one candidate:

$$\begin{array}{cccc} & 0 & 1 & 2 & 3 \\ 0 & [P_1]_0 & [P_2]_0 & [P_3]_0 & [F_i]_0 \\ 1 & [P_1]_1 & [P_2]_1 & [P_3]_1 & [F_i]_1 \\ & \ldots & & & \end{array} \qquad (1)$$

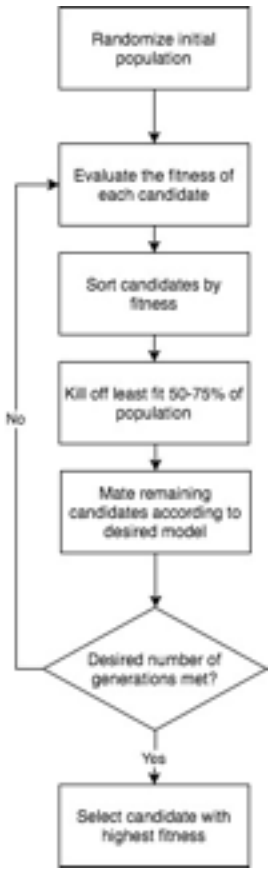

Figure 1. This flowchart shows the general structure of the GA used. The full code can be found in the appendix of this paper.

Parameters 1 and 2 (P1 and P2) represent the C-O bond lengths of the molecule, and were randomly and uniformly drawn from the range 1.0-2.0 Å, a reasonable interval given that the observed bond length is 1.16 Å (Phan et al., 2003). The third parameter represents the bond angle between the two C-O bonds, in the range of 0° to 180°. Finally, the fitness of each candidate was calculated using these same three parameters. The fitness function is based on the electrostatic potential between each charge pair permutation and follows the equation:

$$F = -k\left(\frac{Q_1 \times Q_c}{P_1} + \frac{Q_2 \times Q_c}{P_2} + \frac{Q_1 \times Q_2}{r_3}\right) \qquad (2)$$

$$r_3 = \left(P_1^{\,2} + P_2^{\,2} - 2 \times P_1 \times P_2 \times \cos(P_3)\right)^{\frac{1}{2}}$$

Where $k = 9 \times 10^9$ N (Coulomb's constant), Q1, Q2, and Qc are the partial charges (d+ and d-) on the two oxygen atoms and the carbon atom. The aim is to minimize the net energy of interaction and thus maximize the fitness score. The negative sign ensures the function is strictly non-negative. The combination of the fitness and sort functions inherently eliminates physically impossible phenotypes produced by not assigning them a fitness score. An example of such a structure is when Parameter 3, the O-C-O bond angle, is zero, such that the oxygen atoms lie on top of one another. This renders the third term of the fitness function undefined, represented in Python as NaN (not a number).

We wrote a mutation function into each mating function to maintain genetic diversity. If a mutation is unfavourable, natural selection will prevent its widespread uptake; otherwise, mutations accelerate the process of evolution. In the absence of mutations, a population risks stagnation - where the population converges on the same non-optimal solution (Johnston, 2003). We used a method analogous to a roulette wheel to incorporate static mutations into our algorithm. Static mutations result in the replacement of a gene with a random new allele (Johnston, 2003). In the mutation roulette, a random number between 0 and 1 is generated for each gene of each candidate. If this number is below the predefined mutation rate, the gene is randomly mutated. For instance, if the mutation rate were set to 0.01, probabilistically, 1% of all genes of all candidates would be mutated per

generation.

Upon the creation of a new generation, the candidates are sorted in decreasing order of fitness using an external NumPy sort function. This allows for the straightforward removal of the bottom-most portion of the population, to be replaced with offspring of the fitter candidates.

Methods

Simulation of Biological Systems

In this section, we will compare each biological system modeled: Alpha, League Monogamy, and Polygamy. In the Alpha model, the top candidate of each generation (index 0) mates with each of the other surviving individuals. This is analogous to breeding systems in wolves and lions (Mech, 1999). The rationale behind this system of evolution is that the alpha individual has the best genes. Thus, the quickest way to improve the fitness of the population as a whole is to spread these genes across the entire subsequent generation.

In the League Monogamy model, the bottom 50% of candidates are removed per generation. Then, potential candidates pair with candidates of similar fitness, producing two offspring. This simulates standard human mating, where status often determines reproductive patterns, and the number of children produced is tending towards replacement (Schmitt, 2005).

To model r-selected polygamy, candidates pair several times per generation, producing one offspring per pairing. Additionally, 75% of each generation is removed to allow for more offspring. As in the real world, the evolutionary benefit of polygamy is that it increases the diversity in the next generation, as each individual can potentially have a unique set of parents (Nutting 1891). Such methods of evolution are often found in r-selected species, such as plants and insects, which must be capable of adapting to drastic environmental changes by reproducing at high rates.

Results

Each evolutionary model was run for 100 generations, after which the highest fitness score was recorded. This was considered to be the optimized solution.

The fitness score to which each final solution was compared was calculated using the same function with experimentally determined data: $P_1=P_2=1.16Å$, $P_3=180°$ (Phan et al., 2003). Table 1 summarizes the solution given by each model, as well as relative fitness scores.

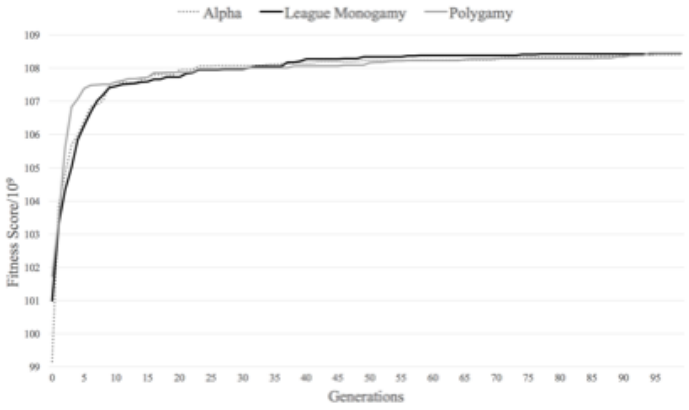

Figure 2. Graph of the highest scoring individual of each generation following the three simulated evolutionary models. The algorithm was run for 100 generations with a fixed mutation rate of 0.1. Standard deviation for each data point is not shown for the sake of visibility; however, the standard deviation obtained for the final fitness scores of the top candidate of each model can be found in Table 1.

Table 1. Optimized data describing the geometry of a CO2 molecule after 100 generations. The relative fitness is the ratio between the calculated fitness score of the ideal known solution and the fitness of the top optimized solution for each breeding system modelled. Therefore, the most accurate model is the one with a relative fitness closest to 1. All values are averages taken from 10 trials. The highest fitness score of each generation is plotted in Figure 2.

Model	Bond Length 1 (Å)	Bond Length 2 (Å)	O-C-O Angle (°)	Final Fitness Score	Relative Fitness	σ in Final Fitness Score (%)
Alpha Male	1.1630	1.163	179.071	1.0841E+11	1.0153	0.12
League Monogamy	1.1608	1.160	179.048	1.0844E+11	1.0155	0.15
Polygamy	1.1636	1.162	179.073	1.0845E+11	1.0156	0.18

Figures 2 and 3 summarize the successive evolution of the highest fitness score of each reproductive model.

Discussion

Figure 2 illustrates the evolutionary pathway followed by each of the three reproductive methods. After 100 generations the curves converge on the same optimized solution, indicating a similar overall efficiency.

However, as highlighted in Figure 3, differences between fitness in earlier generations shows that each breeding system has distinct characteristics. For example, r-selected polygamy appears to be initially the most efficient, due to the rapid initial improvement in population fitness. This correlates to the biological rationale for polygamy, that it allows the population to adapt very quickly to drastic changes in the environment, such as starting from an entirely random molecular geometry (Gould & Lewontin, 1979). However, because the 'best' genes do not dominate the gene pool, the polygamy model experiences the longest plateau before reaching the optimized solution. This suggests that it may not be necessary for an r-selected species to reach an idealized genome. By definition, r-selected species thrive in environments of constant changing pressures, where the requirements for the 'perfect' genome can change regularly. This highlights an inherent limitation of this system, one that makes it unfavourable for species in more stable environments, such as humans (Gould & Lewontin, 1979). It is also worth noting that the results of the polygamy model showed the greatest standard deviation; 0.18%, suggesting a general instability in this evolutionary system.

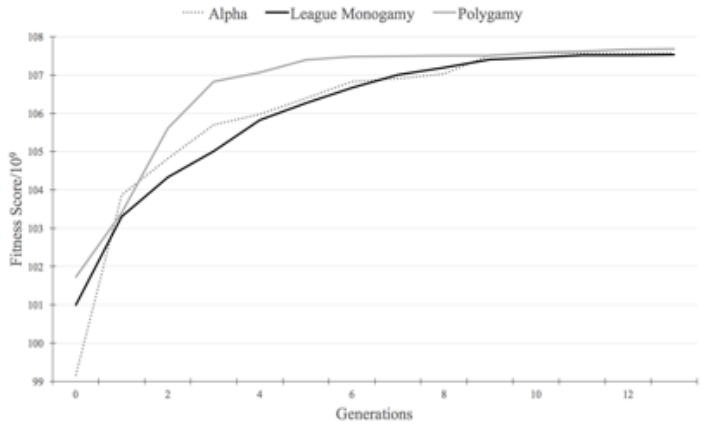

Figure 3. Graph of the highest scoring individual of each generation following the three evolutionary models simulated, for the first 14 generations. As in Figure 2, the standard deviation for each data point is not shown for the sake of visibility.

Similarly, the Alpha Male model displays a sharp initial increase in fitness, as the genes of the top candidate flood the gene pool. However, this results in very low genetic diversity in subsequent generations, risking stagnation. This can be prevented with a sufficiently high mutation rate; however, of the three models, the Alpha model is still most susceptible to achieving false or local maxima, as illustrated in Figure 4. Nevertheless, because of the low genetic diversity, the uncertainty associated with the results of this model is lowest: ±0.12%. In sum, the Alpha model will give the most precise solution, but is best used when an approximate solution is already known. It is important to note that such a method for reproduction is generally only seen in k-selected species, such as lions and wolves, whose environmental pressures remain comparatively stable, meaning they can afford to have relatively low genetic diversity (Huston, 1979).

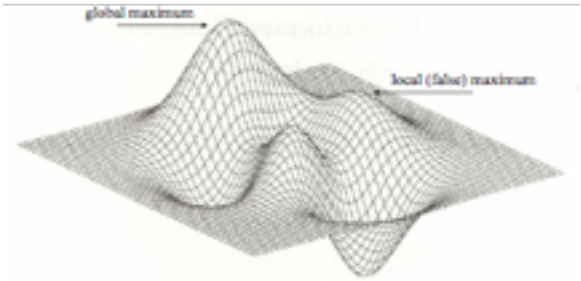

Figure 4. This generalized fitness landscape demonstrates the concept of a local maximum in comparison to a global one. The z-axis represents fitness, while the xy plane represents two parameters. Getting "stuck" at a local maximum is one of the biggest dangers for genetic algorithms, as it can lead to very imprecise and inaccurate results. One of the best defenses against this is high rates of mutation. Adapted from the work by Clegg (2008) without permission (13).

League monogamy is interesting in that it most closely approximates human mating systems. Because it has the most moderate rate of evolution, we posit that it is the most widely applicable model. It is worth noting that this model contains the technically simplest code, which can be useful when approaching a problem that is otherwise complex. However, because of the gradual rate of evolution, it may be inefficient when considering a problem for which the candidate solutions are described by a large number of parameters.

Limitations

We did not design our genetic algorithm to comprehensively simulate biological systems, meaning there are limits to the generalizability of its results. For instance, there was no regulation of successful offspring breeding with their parents. For our purposes, this may be beneficial to the evolution of the population, as both the offspring and parent may be leading

candidates in the race to optimization. However, in biological systems, inbreeding can introduce unfavourable mutations, causing offspring to have lowered fitness or even to die.

Furthermore, in the algorithm proposed here, there was no maximum lifespan, meaning that a high-scoring candidate could maintain its position in the population for many generations, something that is not possible in the biological world.

One of the main limitations of our fitness function was in its physical accuracy. For example, the function did not account for the screening effects of carbon on oxygen, which would have made the 180° structure vastly more favourable. In order to eliminate this effect, a specific if loop was written for candidates with P3=180°, such that their fitness was evaluated according to the following equation:

$$F = -k\left(\frac{Q_1 \times Q_c}{P_1} + \frac{Q_2 \times Q_c}{P_2}\right) \quad (3)$$

This function assumes no oxygen-oxygen interaction due to the screening effects of carbon. Even so, partial screening is not accounted for.

Finally, we note that the final fitness score does not change dramatically in response to large changes in bond angle, which is untrue in experimental settings (Wu & Chern, 1997). This is likely because the derivative of cos(x) is -sin(x), which approaches 0 as x nears the observed bond angle of 180°. This means that with an angle close to 180°, the rate of change of the cos(x) term is very small, even for large changes in x.

Conclusion

This experiment is important not only in the pursuit of the most efficient genetic algorithm, but also to further our understanding of why different species and populations have developed such a diverse range of reproductive strategies: how does each mode affect evolutionary speed? In what ways does this serve the population?

As might be expected, each system of evolution confers advantages and disadvantages to the population upon which it acts. Models reliant on extreme genetic diversity and rapid reproduction, such as the polygamy model, are best suited for determining an approximate solution, as it retains the greatest uncertainty, but takes the least amount of time to run, and explores the largest number of candidate solutions. Conversely, problems that require high precision, and for which the solution is already approximately known, would best be solved by a lower diversity model such as Alpha. We posit that League Monogamy is the most generally applicable model, as it is both relatively efficient and accurate. Furthermore, because it is the simplest to program, the model would be particularly efficient to use on highly complex problems involving many parameters and a sophisticated fitness function. For this particular optimization problem, any evolutionary system could be used to solve the problem in under 100 generations.

Future work could explore the trends discussed above in a more complex optimization problem. This would allow us to see how significant the differences in global maximum uncertainty and fitness function dynamics can be, and whether they might give rise to a preference for one system over the others.

Acknowledgements

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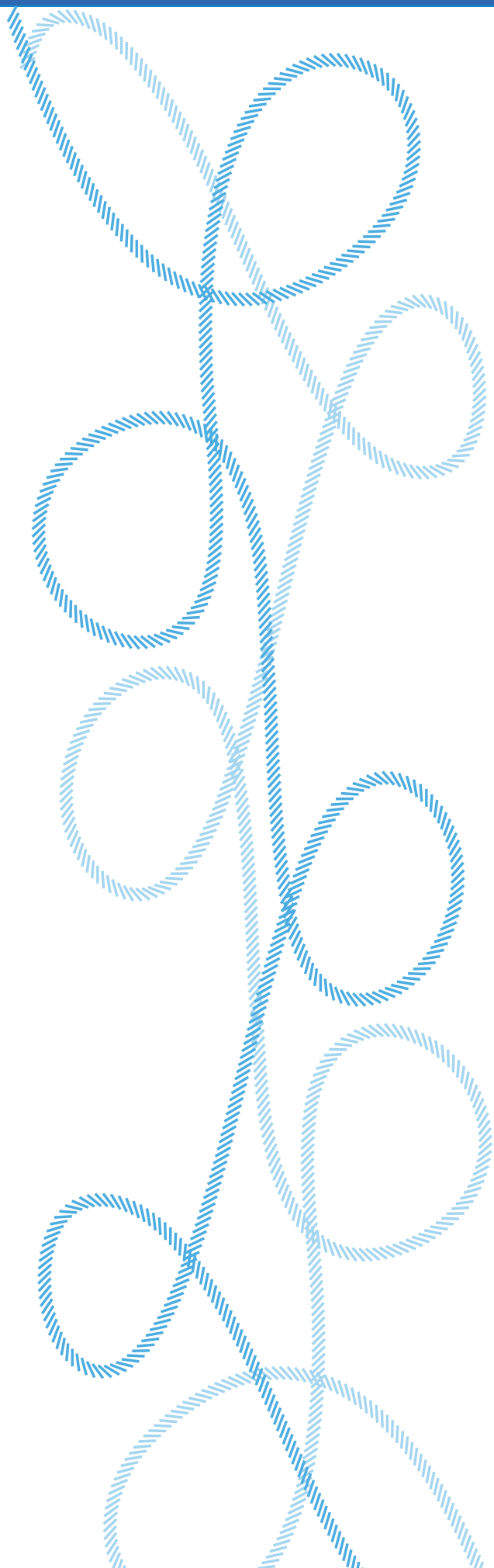

Exploring the role of e-mental health services in Canadian mental health care: A Review

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Abstract

Barriers to conventional treatments for mental health concerns in Canada posit the emergence of information and communications technology (ICT) as a means of providing mental health care; this is widely referred to as e-mental health care. Such platforms currently provide a myriad of mental health services in four broad categories: information dissemination, screening and assessment, intervention and peer support. Coupled with further research, careful consideration of e-mental health models in other countries and current barriers can be used to refine pre-existing e-therapy approaches.

Introduction

An emerging body of evidence implicates the use of digitized and Internet-based interfaces to address mental health concerns. In recent years, e-mental health services have garnered increased speculation to meet the escalating onset of mental health issues, in addition to the increasing demands for localized and easily accessible interventions (Reynolds, Griffiths, Cunningham, Bennett & Bennett, 2015). Although a uniform and unanimous definition does not yet exist, e-mental health can be most accurately described as a broad, umbrella term encompassing a range of information and communication technology (ICT) based interventions (Mental Health Commission of Canada, 2014). ICT refers to media that enable communication and information exchange; it includes the Internet, wireless networks and mobile devices, among others (Mental Health Commission of Canada, 2014). Specifically, e-mental health services can be delivered through a large array of technologies such as e-mails, web-platform, websites, social media, instant messaging, cell phones, virtual realities, video-conferencing, and games (Wozney et al., 2017).

It is estimated that mood disorders, anxiety disorders, schizophrenia, attention deficit/hyperactive disorder (ADHD), conduct disorders, oppositional defiant disorders (ODD), substance use disorders, or dementia will impact 20% of Canadians every year (Hind, Sibbald, 2015). However, only 33% of these individuals receive appropriate treatment (Hind & Sibbald, 2015). In fact, in 2013, Statistics Canada reported that 500,000 Canadians miss work due to a mental health concern (Bradeley & Wang, 2017). It is also important to note that this figure does not encompass the individuals who are unemployed due to their mental health, and others who simply call in sick because they do not feel comfortable explaining the real mental health concern (Bradeley & Wang, 2017). By the year 2041, mental health issues are predicted to negatively impact the quality of lives for over 8.9 million Canadians (Hind & Sibbald, 2015). Moreover, many studies have established several barriers associated with face-to-face treatment for certain geographic, ethnic and low socioeconomic populations. Advancements in technology have opened doors to e-mental health care, allowing effective interaction and engagement between individuals deprived of such services and their health care providers (Mental Health Commission of Canada, 2014). Examples of technologies that permit such accessible mental health interactions include e-health records, telemedicine, web based screening, videoconferencing and online training (Mental Health Commission of Canada, 2014). The increasing role of technology in the lives of Canadians further validates the use of information and communication technology (ICT) to facilitate e-mental health care delivery (Mental Health Commission of Canada, 2014). Today, of the 22 million Canadians that use mobile devices,

62% are smartphone users (Mental Health Commission of Canada, 2014); this further validates the use of information and ICT as a viable and effective medium to facilitate e-mental health care delivery.

Currently, e-mental health models are mainly used for adult populations suffering from depression and anxiety. Such services facilitate knowledge dissemination, assessment and screening, interventions and peer support (Las & Adair, 2014). Countries such as Norway and Sweden have already implemented Internet-based mental health services through websites created by the general public, professionals, or voluntary organizations for issues ranging from emotional, cognitive, and/or relational problems to addiction and abuse (Andersen & Svensson, 2013). Other countries such as Australia have also utilized e-mental health services as an intervention strategy for Australians experiencing mental illness (Australian Government, 2012). One of its most prominent e-mental services includes "eheadspace," a youth-focused telephone and online counselling service for individuals between the ages of 12 to 25 (Australian Government, 2012). Australia also piloted "Virtual Clinic", an online national counselling service offering physician prescribed self-help courses to alleviate mild to moderate symptoms of anxiety and depression (Australian Government, 2012). Additionally, Australia recently established an e-mental health portal that acts as a search tool to provide users with a plethora of information and resources regarding various conditions, as well as online and crisis support services (Australian Government, 2012). Moreover, the National Institute for Health and Care Excellence (NICE) guidelines at the United Kingdom have recently commended the use of computerized cognitive behaviour therapy (CBT) as a first-line treatment for individuals afflicted with mild to moderate depression (National Institute for Health and Clinical Excellence, 2005). Furthermore, a study was conducted in Spain to evaluate an ecological momentary assessment web application known as MEmind (Bonal, Barrigon, Carballo & Baca-Garcia, 2016). The application was presented to 13883 patients, however, it was actually utilized by 2842 patients (Bonal, Barrigon, Carballo & Baca-Garcia, 2016). Women, young individuals, and those with neurotic disorders are the main populations that used MEmind (Bonal, Barrigon, Carballo & Baca-Garcia, 2016). Strategic planning for the implementation of e-Mental Health has also commenced in the United States as a means of providing e-therapy for substance abuse (Mental Health Commission of Canada, 2014). The success of e-mental platforms in Norway, Sweden, Australia, and the United Kingdom serves provides compelling evidence for the provision of e-mental health services in developed countries such as Canada; both as an alternate mode of therapy and as an adjuvant to pre-existing treatment regimes.

Types/Overall Structure

Delivery of e-mental health care can be classified into one of two broad categories: knowledge dissemination and the use of specific technologies to augment or better facilitate interventions, diagnosis and monitor prognosis (Las & Adair, 2014). Provision of mental health information allows for empowerment via better articulation of one's needs, as health care providers are no longer perceived as the sole resource for acquiring a comprehensive understanding of one's conditions (Mental Health Commission of Canada, 2014). For instance, online platforms such as www.whatworks4u.org allow users to share personal thoughts and experiences regarding the efficacy of specific treatment options (Mental Health Commission of Canada, 2014). Such platforms allow for individuals to play an active role in determining their treatment regimes, and thus serve a crucial step towards patient centered and culturally contextualized care (Mental Health Commission of Canada, 2014).

Specific technologies refer to a broad group of services and applications that directly provide e-mental health care (Mental Health Commission of Canada, 2014). Smartphone applications are emerging as a novel means of e-mental health care due to positive experiences by patients and doctors and willingness to use (Hind & Sibbald, 2015). Coupled with the increasing accessibility of mobile devices by younger generations, such interventions are especially effective for youth populations, as they have an increased likelihood of being affected by mental health concerns (Hind & Sibbald, 2015). Examples of mental health applications include PTSD Coach, which allows users to access information about PTSD, coping mechanisms, or track their symptoms (Anthes, 2016). Other apps include FOCUS for assessing the moods of schizophrenic individuals, ClinTouch for informing a clinical care team if an individual is experiencing relapse, and Ginger.io, which connects users with

a mental-health coach and monitors their regular activity, notifying them if there is an alarming change in their patterns (Anthes, 2016).

Computerized interventions consist of text based and video modules to deliver cognitive behaviour therapy sessions (Mental Health Commission of Canada, 2014). MoodGym and Beating the Blues are two web based platforms that deliver such e-mental health care (Mental Health Commission of Canada, 2014). Websites are also another medium that effectively addresses mental healthcare needs. One example is a Canadian website, eMentalHealth.ca, which provides information about mental health services such as nearby organizations catering to the individual's needs, information sheets, screening tools, mental health events, latest mental health news, as well as ongoing research studies on mental health (eMentalHealth.ca). Wearable gadgets that detect fluctuations in baseline physiological measurements and lifestyle trends are also increasingly being used to ensure recovery symptoms and report relapse signatures.2 Quantitative data conveying electrocardiograms, sleep patterns, diet and exercise patterns as well as light sensitivity as a predictor of mood is currently used within the geriatric population (Mental Health Commission of Canada, 2014). Virtual reality e-mental health care interventions consist of 3D online environments that allow users to navigate real life scenarios and situations (Mental Health Commission of Canada, 2014). Additionally, such models are useful in stimulating environments that may be difficult to replicate in real life; for instance, war field environments for the treatment of soldiers afflicted with PTSD (Mental Health Commission of Canada, 2014). Peer support e-mental networks utilize crowdsourcing to allow for anonymous, peer to peer support (Mental Health Commission of Canada, 2014). For example, Big White Wall specifically addresses individuals afflicted with psychological distress by allowing for peer-to-peer interactions via webcam, audio and instant messaging (Mental Health Commission of Canada, 2014). This allows individuals going through similar conditions to engage in mutual support and share experiences (Mental Health Commission of Canada, 2014).

Robots that mimic animal behaviour and 'health bots' are the two types of robots used as therapeutics for mental health concerns (Mental Health Commission of Canada, 2014). The former plays a large role in palliation and facilitating relaxation, as such technologies incorporate/administer the documented therapeutic effects of animal therapy (Filan & Llewellyn, 2006). Health Bots refer to a larger class of robots that perform pre-programmed structured tasks such as delivery of speech therapies, reminding patients to take medications, as well as assisting individuals with memory and speech impairments (Mental Health Commission of Canada, 2014). Gaming, as a mental health intervention, prompts the development of cognitive behavioral skills, self-awareness and ability to address undesired thoughts and situations (Mental Health Commission of Canada, 2014). The widely popular, Sparx (www.sparx.org.nz), provides such therapeutic potential by prompting users to combat negative automatic thoughts (NAT) and overcome problematic situations, as part of their quest on an imaginary island (Mental Health Commission of Canada, 2014).

Advantages and Disadvantages

Overall, e-mental health services offer many advantages to individuals seeking treatment; both in comparison to conventional face-to-face treatment and as an adjuvant to pre-existing regimes. The anonymous nature of such therapies allows individuals who fear social interaction to seek support without judgement (Chan, Farrer, Gulliver, Bennett & Griffiths, 2016). Anonymity may also allow individuals otherwise hindered by cultural beliefs to receive support (Chan et al., 2016). Moreover, the form of therapy curtails the temporal and geographic barriers such as meeting specific business hours and travelling large distances to receive treatment (Chan et al., 2016; Choi, Sharpe, Lee & Hunt, 2013). Furthermore, this allows individuals to overcome financial barriers, due to a reduction in transportation costs and the significantly lower costs associated with online therapy (Chan et al., 2016). Additionally, on a macroscopic scale, e-mental health services are cost effective and feasible in terms of delivery to a large population (Chan et al., 2016; The Royal Australian College of General Practitioners, 2015). Furthermore, in comparison to the average waiting time of 20 weeks to see a psychiatrist upon referral, the low waiting times of e-mental health services address mental health concerns in an efficient and timely manner (The Royal Australian College of General Practitioners, 2015; Barua & Ren, 2016). This also allows professionals to allocate their time and resources to individuals with a

severe mental illness, for whom e-mental health care is not a viable option (The Royal Australian College of General Practitioners, 2015; Barua & Ren, 2016). Consequently, this may also be advantageous to individuals suffering from physical incapacity or anxiety (The Royal Australian College of General Practitioners, 2015).

However, e-mental health services also pose many barriers to effectively meet the mental health care needs of its designated population. For instance, individuals with brain injuries and loss of motor control may lack the ability to use such services (The Royal Australian College of General Practitioners, 2015). In fact, a study revealed that adults with a chronic mental illness were less likely to have access to an Internet connection (The Royal Australian College of General Practitioners, 2015). The lack of confidentiality and privacy that may be associated with computerized health records is also a common concern (Chan et al., 2016). Difficulty in accessing the large amounts of information available online, as well as ensuring its quality and accuracy is a valid concern regarding e-mental health services that facilitate knowledge dissemination (Chan et al., 2016). Furthermore, current e-mental interventions do not address attrition related to such services, as a result of drop-outs and lack of follow-ups (Chan et al., 2016). Moreover, an online medium of expression may not allow individuals to reveal underlying emotions in a wholesome manner (Chan et al., 2016). Acceptability of e-mental health interventions is also a major barrier, as several studies reveal that many individuals prefer in-person mental health care (Chan et al., 2016). This may be due to the novelty of e-mental health care models, and the lack of knowledge regarding the effectiveness of such services (Chan et al., 2016).

Future Directions

As evident, e-mental health care has vast implications for transforming mental health care delivery in Canada. Future research should be targeted towards developing methods to integrate e-mental health care into the pre-existing therapies (Mental Health Commission of Canada, 2014). Additional randomized controlled trials and comparison trials with face-to-face therapeutics should be conducted to further validate its therapeutic potential, as the current evidence is sparse. These trials will also serve as further evaluations to confirm whether or not the benefits outweigh the disadvantages of e-mental health care. Novel lines of investigation should also explore its effectiveness within various demographics, delivering culturally contextualized care, and its ability to prevent attrition rates. Furthermore, studies should be directed towards catering e-mental health care approaches towards individuals lacking accessibility to required technology and further expanding this model to third-world countries. Additionally, strict guidelines should be established to prevent concerns relating to ethics, patient confidentiality, and legal issues (Mental Health Commission of Canada, 2014). Lastly, because e-mental health is one branch of e-health services, future studies should evaluate the use of technology in specialties outside mental well-being such as physical and social well-being (Mental Health Commission of Canada, 2014). Examples of such services can include the introduction of e-fitness to promote a healthy lifestyle, and e-care, which would provide individuals with online access to medical professionals for health assessment and information about their medications. Given the fast-paced rise in technology and the number of Canadians that are utilizing such technologies, the reviewed advantages of e-therapy support the development of a novel nationwide strategy to address mental health care, as well as other categories of e-health.

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Comparison of Discourse Surrounding CRISPR/Cas9 in the Media and Peer-Reviewed Literature

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Abstract

Since its development in 2013, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cas9 gene-editing technologies have dramatically impacted the field of genetics research. CRISPR/Cas9 has received a lot of attention in the news in recent years, and accurate portrayal of this technology by the mainstream media has the potential to shape its perception by the public in a way that is conducive to its possible implementation as a viable tool for genetic engineering. Our aim was to evaluate how the discussion of CRISPR/Cas9 in the mainstream media reflects and compares to that of the academic literature. We surveyed mainstream news articles (n=60) and scientific review articles (n=30) that discussed CRISPR/Cas9. Using an a priori coding scheme, we found that while the news does not accurately reflect the current state of CRISPR/Cas9 research and development, it provides more perspectives and considers broader social implications compared to the academic literature. Therefore, both news media and academic papers provide valuable contributions to the conversation but news articles in particular have the opportunity to improve the accuracy or thoroughness of their coverage on the topic.

Introduction

The ethical responsibility of public communicators of science has come under increasing scrutiny over the past years. Previous studies by Kamenova (2015) and Racine (2010) have shown that the media provides overly optimistic depictions of developing biotechnologies, emphasizing benefits over risks and fostering unrealistic expectations for the speed of application. We sought to analyze this type of reporting in the case of CRISPR/Cas9, a gene-editing technique that has had a profound impact on the field of genetic research in recent years. Compared to its alternatives, such as TALENs (Transcription Activator-Like Nucleases) and ZFNs (Zinc Finger Nucleases), and Meganuclease, CRISPR offers clear cost advantages, being three to six fold cheaper per reaction (Samy, 2017). It has therefore provided a more accessible and efficient method of editing DNA.

While CRISPR/Cas9 has gained increasing attention both in mainstream media and in academic literature, to our knowledge, little research has been done that compares and analyzes the discussion on different platforms. Given CRISPR/Cas9's potential to impact both genetics research and society as a whole, we believe it is important that there is transparency among scientists, the mainstream media, and the general public regarding its major developments. Our aim was to evaluate how the discussion of CRISPR/Cas9 in the mainstream media reflects and compares to that of the academic literature. Results from our study can give us an understanding of the similarities and differences between expert and public discussion on the topic of CRISPR/Cas9 and gene editing more broadly.

Method

To compare the discussion of CRISPR/Cas9 in academic and non-academic sources, we conducted a scoping review consisting of the keywords, "CRISPR Cas9 human review", within the time frame of 2005 to 2017. We used PubMed as the search database for our academic sample and Google News for our non-academic sample. Despite the substantial amount of work that is currently being done with CRISPR/Cas9 in both human and non-human models, we focused on human applications of the technology due to its relev-

ance to the public. From our search, we retrieved 280 unique peer-reviewed articles from PubMed and 215 media articles from Google News. For the academic sample, we included only peer-reviewed articles that primarily focused on human applications of CRISPR (n = 187). From the 187 articles for the academic sample and 160 articles from the non-academic sample remaining, we randomly selected 30 academic articles and 60 non-academic articles. We ensured that each article was reviewed in its entirety, and not just specific sections of it.

We generated a coding frame informed by Benjaminy, Lo, and Illes (2016) based on categorical variables representing tone, portrayal of the technology's salience, and discussion of the following elements: controversies, societal risks, applications, future projections, and engineered humans (Table 1). For tone, we considered the author's stance on the topic, which was coded as negative, neutral, or positive. For salience, we considered how strongly the author suggests the importance of the technology, which was coded as unimportant, expected, important, or revolutionary. In particular, "expected" indicates that the technology is portrayed as along the expected pace of scientific advancement, and "revolutionary" indicates that it is portrayed as a complete or dramatic change. Next, we considered whether controversies were discussed, whether societal risks were discussed, whether applications were discussed mainly in research or clinical contexts, whether future projections were the main focus of the article, and whether there was mention of engineered humans. We consider research applications to include the study of molecular genetics and human diseases, whereas clinical applications include germline editing, treating and curing diseases, and improving medical technology.

Coding of the articles was done by all three researchers, who were trained by coding a few articles separately, then discussing and resolving any disagreement. After training, the academic and non-academic samples were each split into three subsets, and each researcher coded one subset.

Finally, after quantitatively assessing each sample, we performed a Chi-Square test for independence to determine whether there was a significant association ($p<0.05$) between each variable and either the academic or non-academic sample (Table 1).

Coding variables	Academic articles (n = 30)	Non-academic articles (n = 60)	p-value
Tone of articles			0.000484
Negative	0%	17%	
Neutral	50%	68%	
Positive	50%	15%	
Discussion of social risks			0.000314
Yes	10%	40%	
No	90%	60%	
Discussion of applications			0
Mainly research	80%	3%	
Mainly clinical	20%	92%	
None	0%	5%	
Mention of engineered humans			0.002060
Yes	10%	50%	
No	90%	50%	
Discussion of controversies			0.00000003
Yes	23%	83%	
No	77%	17%	
Focus of article: Future projections			0.00000052
Yes	3%	58%	
No	97%	42%	
Salience			0.42
Unimportant	0%	3%	
Expected	50%	38%	
Important	43%	48%	
Revolutionary	7%	10%	

Table 1 Summary of main coding variables and p-values yielded from Chi-Square tests

Results

Six of the seven variables assessed showed a significant association between the variable and the type of source they were represented in: tone, discussion of controversies, discussion of societal risks, discussion of applications, discussion of future projections, and mention of engineered humans. In our news media sample, the majority of the articles (68.3%) depicted human applications of CRISPR/Cas9 in a neutral tone while only a small portion used negative (16.7%) or positive tones (15%). In contrast, the tone of the academic articles had an even divide between neutral (50%) and positive (50%) tones.

Societal risks were discussed more often in news articles (40%) than in academic articles (10%). Similarly, a higher percentage of non-academic sources (83%) discussed controversies of CRISPR/Cas9 in human applications compared to academic articles (23%).

There was also a significant difference in how academic and non-academic articles portrayed applications of the technology—while most non-academic articles (92%) focused on clinical applications, most academic articles (80%) focused on research applications. We also observed a significant difference in the

mention of engineered humans between the two types of sources with half of the news articles (50%) mentioning engineered humans whereas far fewer academic articles (10%) did the same.

In terms of the future projections of the technology, the vast majority (96.7%) of the academic articles provided no claims for the future of CRISPR/Cas9, while they were brought up in more than half (58.3%) of the non-academic articles.

Conclusion and Discussion

In our research, we found statistically significant differences between article sources in six of the seven topics that we investigated. Overall, the news media's discussion of applications and future projections was not reflective of the discussion in the academic literature. As we expected, in academic articles, CRISPR/Cas9 is still being discussed almost exclusively in the research context. On the other hand, the media focuses largely on usually futuristic applications to humans. More specifically, academic papers often discuss CRISPR/Cas9's application to the study of human diseases and molecular genetics, while news articles discuss the possibility and implications of using CRISPR/Cas9 for germline editing, treating and curing diseases, and "engineering" humans. Similarly, the majority of media articles discuss future projections of CRISPR/Cas9 as its main subject, while academic articles rarely do, focusing on present work instead.

Furthermore, we found that the news media is more attentive to the societal risks and controversies involved with this technology, including policy guidelines for CRISPR/Cas9's continued development and ethical controversies surrounding germline editing. Since it is not necessarily the responsibility of researchers to answer this question, it is valuable that the news media creates a forum for this discussion. In general, the greater distribution of tone in news media indicates that it may offer more perspectives.

Future studies could determine what factors cause the differences found in the discourse surrounding CRISPR/Cas9 in the academic literature and news media. A better understanding of these factors would provide insight into how scientific research on CRISPR/Cas9 is translated by the mainstream media. Learning to reduce or control these factors could promote more scientifically and socially responsible communications about the promises and limitations of CRISPR/Cas9. This has the potential to shape the understanding of the public, which could then aid the successful implementation of CRISPR-based technologies.

News media and academic articles have differing perspectives and information to offer. As we expected, academic literature provides a thorough account of CRISPR/Cas9 research and development that is being done. While the news does not accurately reflect the current state of this research, it brings in more perspectives and considers broader social implications. Therefore, it is important for expert and non-expert perspectives alike to be involved in the conversation. At the same time, news articles in particular have the opportunity to improve the accuracy or thoroughness of their coverage on the topic. Accurate portrayal of this technology by the mainstream media has the potential to shape its perception by the public in a way that is conducive to its possible implementation as a viable tool for genetic engineering.

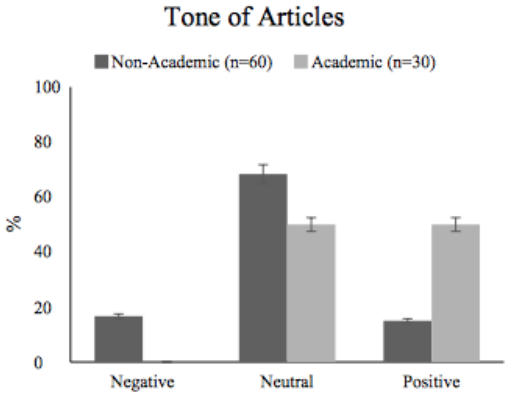

Fig. 1 Tone of discussion represented in non-academic (n = 60) and academic (n = 30) articles

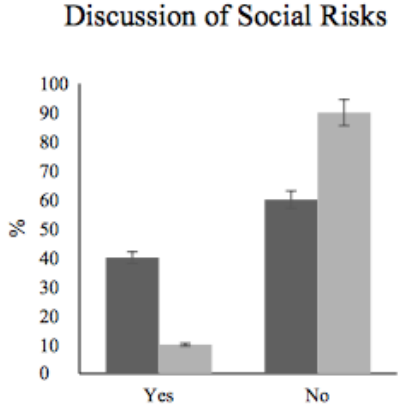

Fig. 2 Discussion of social risks in non-academic (n = 60) and academic (n = 30) articles

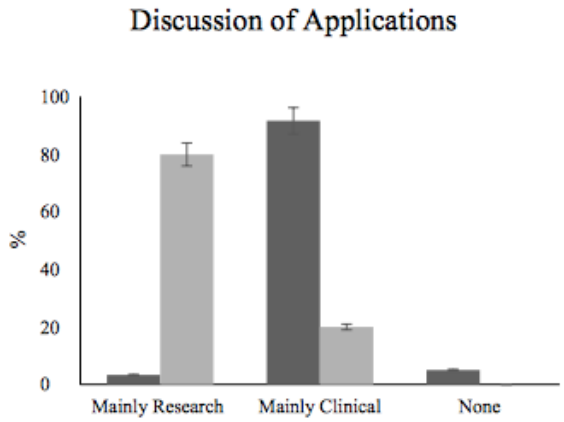

Fig. 3 Discussion of applications in non-academic (n = 60) and academic (n = 30) articles

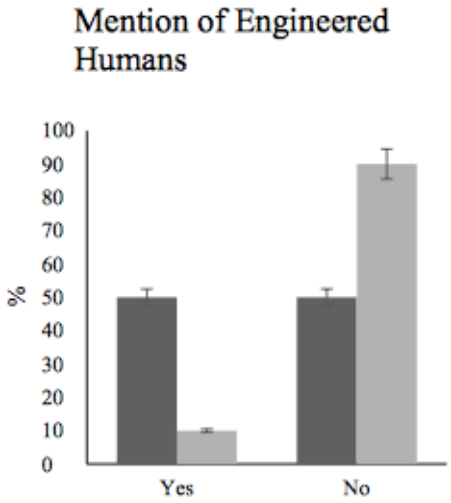

Fig. 4 Mention of engineered humans in non-academic (n = 60) and academic (n = 30) articles

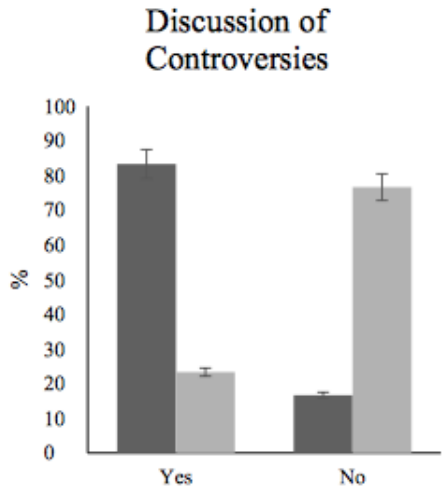

Fig. 5 Discussion of controversies in non-academic (n = 60) and academic (n = 30) articles

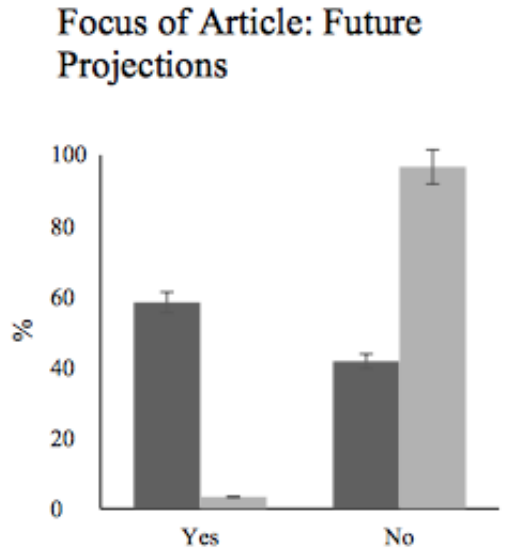

Fig. 6 Future projections as the focus of discussion in non-academic (n = 60) and academic (n = 30) articles

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Use of BioID to detect protein-protein interactions

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Abstract

Proximity-dependent biotin identification (BioID) is a novel approach to identify protein-protein interactions (PPIs) in a natural cellular environment. BioID exploits a mutant form of a biotin protein ligase found in Escherichia coli, BirA*, that promiscuously catalyzes biotinylation of proteins in close-proximity of the enzyme. Biotinylated proteins are then purified with conventional methods. BioID has been shown to overcome many of the limitations faced by traditional PPI techniques, such as co-immunoprecipitation, proximity ligation assays and yeast two-hybrid systems. The main advantages of BioID as compared to these methods include high sensitivity and spatial resolution, preservation of physiologically- relevant conditions, and detection of weak or transient interactions. Despite some inherent limitations, BioID remains a promising PPI technique and has led to more advanced methods, such as BioID2 and split-BioID.

Introduction

For decades, the genome revolution has contributed to our understanding of human disease, however proteomics has contributed greatly to the field as it allows for large-scale analysis of proteins in order to understand functions of genes implicated in disease. In addition to their complexity, proteins rarely exist in simple complexes and often form interconnected networks in order to exhibit important functions as part of a larger mechanistic pathway. Therefore, understanding their function in the context of protein-protein interactions (PPIs) can provide great insight into the numerous mechanisms underlying disease. Many of the approaches currently used to assess PPIs are done so in an environment different to that in which they naturally occur and often lack the ability to detect weak or transient interactions. Therefore, many proteins remain unrepresented by these conventional methods of screening for PPIs. Here, we discuss an innovative approach for PPI screening called BioID, and highlight the key reasons for its superiority to these conventional methods.

Limitations of common PPI techniques

One of the most widely used techniques for detecting PPIs is co-immunoprecipitation (co-IP), which is often used to test whether two known proteins interact, or to screen for novel protein interactors of a protein of interest (Golemis, 2002). Co-IP harnesses the principle of a specific protein-antibody reaction, in which an antibody against the protein of interest, conjugated to beads, is used to precipitate the protein of interest and its interactor proteins. Subsequent western blotting or mass spectrometry analysis can be performed to blot for a known protein, or to identify novel interactors, respectively. Although this technique allows protein interactions to occur in non-denaturing conditions, if the protein interaction or antibody is weak, certain interactions are often not detected (Golemis, 2002).

Another common PPI screening technique is called a proximity ligation assay (PLA), which uses two primary antibodies raised in different species for two specific proteins of interest (Lin et al., 2015). Secondary antibodies, each conjugated to an oligonucleotide (PLA probe) bind to the primary antibodies and a ligation solution containing two oligonucelotides and a ligase is added. If the proteins are in close proximity, the oligonucleotides in the solution will hybridize to 2 the PLA probes to form a closed circle. Subsequent addition of an amplification solution allows for circular polymerase chain reaction amplification, resulting in a fluorescent signal (Lin et al., 2015). Although this technique allows for visualization of PPIs in

physiologically-relevant conditions, it is not appropriate for large-scale interaction screens (Lin et al., 2015).

The last commonly used technique for PPI screening is the yeast two-hybrid (Y2H) system, in which the interaction between two proteins, the bait and prey, activates a reporter gene that allows for growth of cells harbouring this gene, on specific media (Brückner et al., 2009). A major drawback for this technique includes its inability to detect indirect PPIs, unless a yeast ortholog to the protein exists to mediate the interaction. Additionally, it harnesses an artificial system in which the bait and prey fused to DNA-binding and activation domains are exogenously introduced into cells and therefore does not allow for analysis of endogenous protein interactions under physiological conditions (Brückner et al., 2009).

BirA* as a promiscuous biotin ligase

Upon discovery of a promiscuous biotin ligase, a novel approach for detecting PPIs was established, termed BioID. Originally, BioID was derived from a DNA-protein interaction screening technique, developed by van Steensel and Henikoff, called DamID. DamID uses the fusion of a prokaryotic Dam methylase to a DNA-binding protein, which when expressed in eukaryotic cells will methylate DNA sequences that are in close proximity. This methylated DNA acts as a marker of the interaction that can be subsequently analyzed (van Steensel and Hanikoff, 2000). BioID, similar to the principle of DamID, uses a biotin ligase, called BirA, fused to a protein of interest, that upon expression in mammalian cells will biotinylate any close- proximity proteins. The biotinylated proteins can then be isolated and identified by traditional methods such as mass spectrometry (Roux et al., 2012).

BirA is a 35-kDa DNA-binding, biotin holoenzyme synthetase ligase originally found in Escherichia coli. In the bacterial system, BirA catalyzes the post-translational modifications of transferring biotin to specific lysine residues of the biotin carboxyl carrier protein (BCCP) subunit of acetyl-coA carboxylase (Beckett et al., 1999). This specific class of enzymes, called biotin-dependent carboxylases, undergo post-translational modification in which biotin is covalently linked to a single lysine residue via an amide bond (Beckett et al., 1999). The biotinylation of these lysine-bearing carboxylases occurs in two steps: (1) BirA catalyzes the conjugation of biotin and adenosine triphosphate (ATP) to form a highly reactive intermediate, biotinyl-5'-adenylate (bio-5'-AMP or biotinoyl-5'-AMP). (2) This activated biotin is retained in the BirA active site until it interacts with a specific lysine residue within a target protein, in which an amide bond is formed between the biotin moiety and the lysine residue (Beckett et al., 1999; Roux et al., 2012). This biotinylation reaction, as shown in Figure 1, is highly specific to biotin-dependent carboxylases, however Roux et al. wanted to obtain a more promiscuous biotin ligase as a tool for detecting PPIs. This led to the discovery of a specific BirA mutant (R118G), called BirA*, which is defective in the self-association and DNA-binding, and displays two- orders of magnitude lower affinity for biotinoyl-5'AMP than the wild-type BirA, therefore resulting in premature release of the highly reactive intermediate (Kwon and Beckett, 2000; Streaker and Beckett, 2006). Furthermore, it has been shown that BirA* expression in E. coli results in promiscuous biotinylation of proteins in close proximity - this was further validated in vitro. This led Roux et al. to further study the potential of BirA* to biotinylate proteins in mammalian cells, which eventually led to its use as a tool for PPIs.

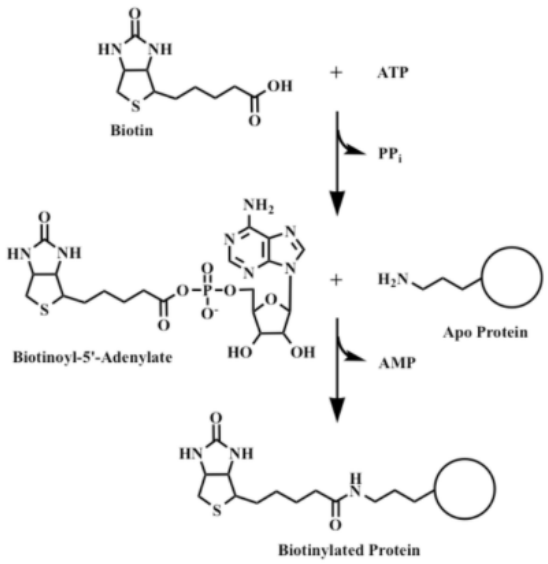

Fig. 1. Biotinylation reaction (Henke and Cronan, 2014). The attachment of biotin to proteins is a two-step process: (1) the biotin ligase, BirA, conjugates biotin and ATP to form a reactive biotinoyl-5'-AMP intermediate, and (2) conserved lysine residues of acceptor proteins, which are nucleophilic, attack the anhydride bond to produce the biotinylated protein.

BirA* function in mammalian systems

To test whether BirA* functions as a promiscuous ligase in a mammalian system, Roux et al (2012) generated myc-epitope tagged BirA-WT (wild type) and BirA*, which they then expressed in HeLa cells. Subsequently, they used streptavidin – which forms an extremely strong non-covalent bond to biotin – conjugated to horseradish peroxidase (HRP), in order to visualize biotinylated proteins via western blot analysis (Roux et al., 2012). This experiment showed that BirA* biotinylated modest levels of proteins as compared with BirA-WT. However, in the presence of 50μm biotin within the HeLa cell tissue culture medium, it was shown that the BirA* promiscuously biotinylated proteins in these mammalian cells (Roux et al., 2012). Therefore, the limiting factor of the level of biotinylation is the concentration of available free biotin, and this biotin must be added in excess to the already present biotin within the fetal calf serum of conventional tissue culture media (Roux et al., 2012). The results of this experiment can be seen in Figure 2.

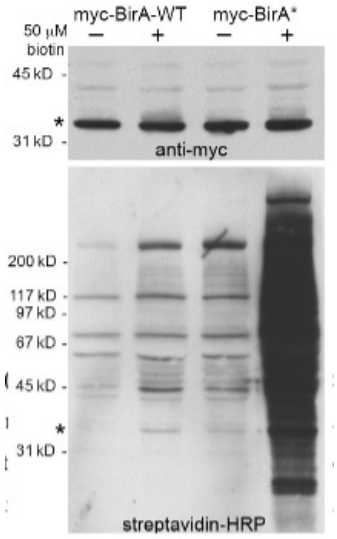

Fig. 2. BirA* enhances biotinylation of proteins in mammalian cells (Roux et al., 2012). HeLa cells were transiently transfected with myc-BirA-WT (wild type) or myc-BirA* (R118G), cultured with or without supplemental biotin (50 μM) and analyzed after 24 hours. Top panel: western blot analysis shows similar levels of the exogenous BirA (asterisk) in samples with anti-myc. Bottom panel: biotinylated exogenous BirA (asterisk) proteins and endogenous proteins were detected with HRP-streptavidin. The myc-BirA* samples showed increased protein biotinylation as compared with the WT isoform (control). This difference is dramatically enhanced by the presence of excess biotin.

In addition to testing the function of BirA* in the mammalian system, Roux et al. (2012) wanted to determine whether BirA* could be used as an experimental tool for identifying close-proximity proteins in vivo. To do this, they generated human kidney fibroblasts (HEK)-293 cells that inducibly express LaA (a constituent of nuclear lamina) N-terminally tagged with myc-BirA*. The cells were subsequently cultured either in the presence (50uM) or absence of exogenous biotin and the biotinylation of endogenous proteins was analyzed via western blot probing with streptavidin-HRP antibody. It was shown, as seen in Figure 3, that the presence of 50uM of exogenous biotin, a large number of nuclear envelope proteins were biotinylated, therefore confirming the biotinylation of close-proximity proteins (Roux et al., 2012).

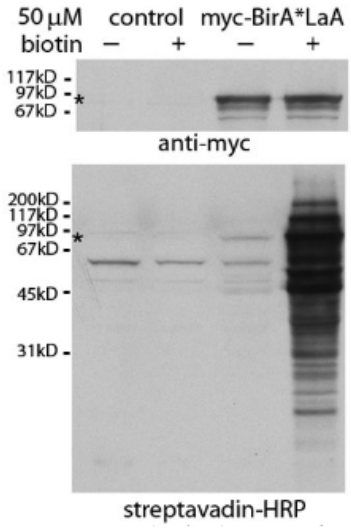

Fig. 3. Proximity-dependent promiscuous biotinylation by BirA*-LaA (Roux et al., 2012). HEK293 cells that inducibly express myc-BirA*LaA or wild-type LaA (controls) were examined after 24 hours of incubation with or without biotin. Western blot analysis of the LaA fusion protein (asterisk) shows detection with anti-myc. The presence of supplemental biotin does not affect the levels of endogenously biotinylated proteins within the control cells. However, this excess biotin significantly increases the biotinylation of endogenous proteins by myc-BirA*LaA.

BioID: novel approach for screening PPI

BirA* is a key player in BioID, a PPI screening technique (Roux et al., 2012). The principle of this system is to fuse a protein of interest to BirA* and subsequently introduce this fusion protein into cells, which are then incubated in excess biotin

(Roux et al., 2013). During this incubation, the BirA* will continuously release the highly reactive molecules of biotinoyl-5'-AMP, leading to biotinylation of any proteins that are in close vicinity of the fusion protein. The biotinylated proteins – direct binding partners and other proteins in close proximity – can then be purified using streptavidin-coated beads (Roux et al., 2013). These beads have a qualitatively high affinity for biotin, forming a non-covalent bond strong enough to withstand extremely harsh lysis conditions. This enables certain proteins (i.e. cytoskeletal proteins) that normally remain insoluble in weaker detergents, to efficiently become soluble and captured (Roux et al. 2013). Therefore, one of the major strengths of BioID is the capacity to maintain important information about PPIs, while utilizing extremely stringent detergents for cell lysis in order to capture proteins such as cytoskeletal proteins (Roux et al., 2013). Upon purification of biotinylated proteins via streptavidin-coated beads, these proteins can be identified by conventional methods such as mass spectrometry, as seen in Figure 4.

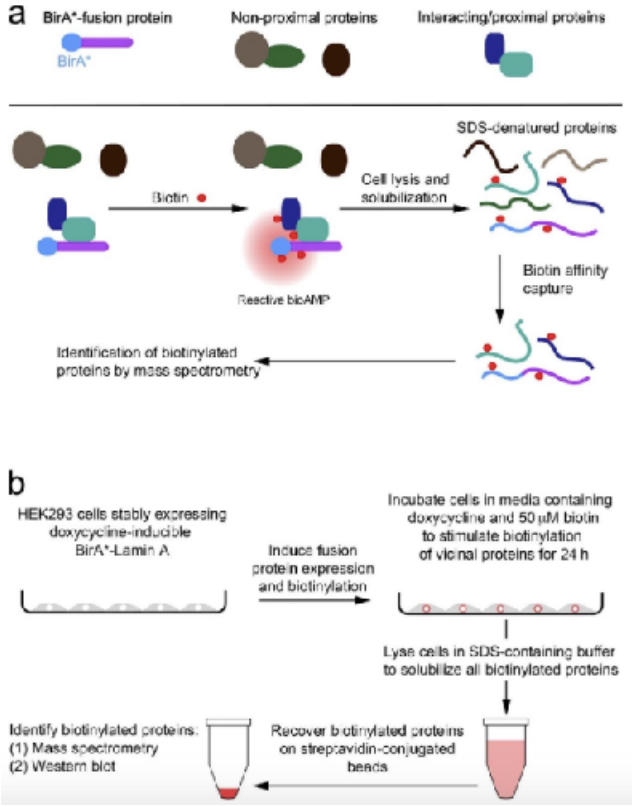

Fig. 4. Model for application of BioID (Roux et al., 2012). (a) Interacting or proximal proteins are tagged and subsequently undergo biotinylation via the promiscuous biotin–ligase, BirA*. Following stringent cell lysis and protein denaturation, affinity purification occurs involving streptavidin beads to pull down the biotinylated proteins. Candidate proteins are often determined by mass spectrometry. (b) Application of BioID to LaA involved the use of HEK293 cells expressing inducible myc-BirA*LaA to test the functionality of BirA* in the mammalian system. Cells were cultured with (50µM) or without exogenous biotin 24 hours before lysis and subsequently lysed under harsh conditions. Streptavidin-conjugated beads allowed for the collection of the biotinylated proteins for later analysis and identification.

Advantages of BioID

Techniques for PPIs, such as PLAs and Y2H systems often face limitations, such that they cannot detect interactions of proteins that contain post-translational modifications or require them for their interactions (Mehus et al., 2015). However, BioID is distinct from these conventional techniques primarily with its ability to detect weak or transient interactions, preserve physiologically-relevant conditions, and identify interactors with high sensitivity (Mehus et al., 2015; Kim, 2016).

Firat-Karalar and Stearns (2015) used BioID to study the protein interactions at the centrosome, translating this work to other researchers who validated the technique's efficacy in screening proximity interactions within mammalian centrosomes. This was due to the strong affinity of biotin for streptavidin, which inevitably allowed for protein purification to occur under stringent denaturing conditions and subsequently preserving the proximal interaction, while solubilizing the centrosome (Firat-Karalar and Stearns, 2015). The direct correlation of conditions promoting protein solubilization and preservation is specific to BioID; often absent in former methods (Roux et al., 2012). Biotinylation precedes solubilization, which allows for weaker and transient interactions to be identified, as outlined by successful detection of BioID-LaA soluble and membrane proteins (Roux et al., 2012).

In non-native environments, which are typically required for PPI techniques such as Y2H systems, proteins and protein fragments are more susceptible to misfolding, relative to their normal cellular context (Roux et al., 2012). However, BioID screens potential vicinal proteins under relatively natural cellular conditions and therefore provides physiologically-relevant information regarding PPIs (Mehus et al., 2015; Kim and Roux, 2016). Although BioID is contingent on exogenous biotin in order for biotinylation to take place

it allows for temporal inducibility of labelling, in experiments where biotinylation process may exhibit a toxic effect.

In addition to its ability to preserve physiological conditions during the point of interaction, BioID allows for detection of biotinylated proteins through the use of streptavidin-coated beads that form strong bonds with the biotin of the modified proteins (Kim, 2016). As such, biotinylation enables the BioID system to reach a maximal level of isolated protein purification. Consequently, this results in greater sensitivity and spatial resolution, as well as low background resolution (Kim, 2016). Additionally, BioID enables detection of low abundance proteins, being a technique that can successfully identify novel PPIs and insoluble constituents that often evade traditional methods (Roux et al., 2012).

Other proximity-labeling techniques, such as selective proteomic proximity labeling assay using tyramide (SPPLAT) and proximity labeling with ascorbate peroxidase (APEX) utilize tyramide-based reagents that covalently attach to aromatic amino acid side chains, such as tyrosine, and hydrogen peroxidase to initiate labeling of neighbouring proteins (Rees, 2015). Sufficient labeling of the protein target is often prevented by formation of constituent aggregation and further impeded by the reagents' properties, as they cannot easily detect individually modified proteins (Rees, 2015). BioID's ability to label lysine residues, which are not only more abundant than tyrosines but also more structurally exposed, circumvents this problem. Furthermore, the labelling frequency when using these tyramide-based reagents is likely to be lower due to its dependency on covalently coupling to aromatic groups, and thus the exposure of these residues. However, tyramide-based proximity labelling techniques perhaps provide even further advantages due to the shorter half life of the tyramide-based reagents as compared to biotin-adenylate ester that are essential for BioID labelling, and thus requires shorter incubation periods (Li et al., 2017). Despite these advantages, BioID is deemed for its simplicity and lack of toxic labeling conditions, as it does not require hydrogen peroxidase to initiate labelling, universal to tyramide-based methods (Branon et al., 2017).

Caveats of BioID

As with any novel technique, comes a number of inherent drawbacks. In any proximity-dependent labeling system, one must consider the accessibility for protein interactions to occur, as the number of proteins, structure and orientation play a vital role in successfully screening these neighbouring proteins (Kim and Roux, 2016). In addition to the inability for BioID to assess the strength of PPIs, the amount of biotinylated proteins is also not a valid means to identify true protein association, as low amounts may be more biologically relevant than those of higher quantities (Mehus et al., 2015; Kim and Roux, 2016; Roux et al., 2012). Furthermore, the protein of interest can also be structurally changed by fusion to BirA*. Independent of the protein's size, the fusion of a tag can affect the protein's structure and stability, alternating its interactions and ultimately its function (Mehus et al., 2015). Additionally, during the irreversible covalent modification of biotinylation of primary amines, site-specific charge loss and alterations of the labeled proteins can arise, which can impair localization and function of the fusion protein as well as vicinal proteins (Kim and Roux, 2016; Roux et al., 2012). This can therefore result in an inaccurate representation of potential interactors. However, this limitation can be overcome by first avoiding alteration of the protein of interest's function by targeting the C or N-terminal regions and subsequently testing the functionality of the final BirA*-fused protein of interest, if the function is known (Roux, 2013).

In addition to the technical limitations of BioID, validation is required to determine whether the protein of interest is directly interacting with the biotinylated proteins or if the detected proteins are merely in the vicinity of the protein of interest. Due to this, there are also several caveats within the interpretation of BioID results. For example, positive interactors that result from the BioID screen do not prove that there is a direct interaction, as labeled candidates may reside in close proximity of the BirA*-fused to the protein of interest, but not physically interact with it (Roux, 2013). Furthermore, false negatives can also arise as a result of true interactors that lack the proximate primary amines required for the biotinylation process (Kim and Roux, 2016).

Finally, biotin is transported into mammalian cell cytoplasms and depends on diffusion into the nucleus (Zempleni, 2005). Despite biotin being non-toxic, the addition of excess biotin as seen in normal conditions, can influence protein function when

incubated for an extended labelling time (Kim and Roux, 2016).

Applications of BioID in other biochemistry techniques

Although the original purpose for developing BioID was to utilize it as a tool for identifying PPIs, its capacity for detecting close-proximity proteins allows for elucidation of meaningful information about protein dynamics and can be combined with other biochemistry tools such as crosslinking and immunoprecipitation followed by high-throughput sequencing (CLIP-seq) in order to unveil different proteomic networks.

In addition, the BirA*-fused proteins of interest can be integrated into the genome of the cell line of interest through homologous recombination via CRISPR-Cas9 genome editing (Ran et al., 2013). By designing homologous arms on either end of the BirA*-protein of interest fusion repair template, the tagged protein of interest can be inserted into the genome of the cells in order to endogenously express these BirA* tagged proteins. The combination of BioID with CRISPR-Cas9 systems provides a mechanism for detecting PPI in physiologically-relevant conditions and thus overcomes this challenge that limits many other PPI techniques.

Future Directions of BioID

BioID2

Kim et al. (2016) recently discovered a more efficient method, termed BioID2. It elaborates on the former system by employing a significantly smaller promiscuous biotin ligase, and in turn efficiently labels close-proximity proteins. Sun2, a type II nuclear envelope (NE) protein, is susceptible to endoplasmic reticulum (ER) mislocalization, like other NE proteins. It does so particularly when its N-terminus is fused to structurally large elements. As depicted in Figure 5a, functional validation of both enzymes showed that BioID2 facilitated appropriate expression of Sun2 at the NE, relative to the ER, at a magnitude of two-fold, in comparison to BioID. This confirms that the reduction in tag size allows for improved functionality. Aside from this, BioID2 possesses a lower optimal temperature and remarkably requires over 15 times less biotin than BioID for promiscuous biotinylation. The introduction of flexible linkers increased the labeling radius of BioID2 to thereby improve identification of proteins, initially refractory to the former BioID system (Kim et al., 2016).

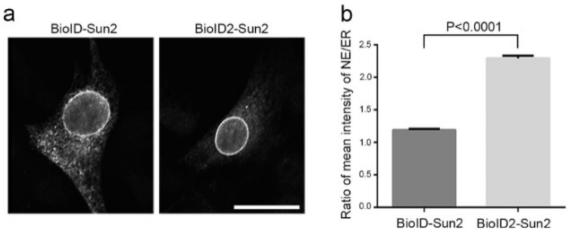

Fig. 5. BioID2 enhances fusion protein localization (Kim et al., 2016). (a) Mouse fibroblast NIH3T3 cells were used to transient express NE protein, Sun2 with BioID or BioID2, marked as BioID-human Sun2 and BioID2-human Sun2, respectively. More appropriate localization was facilitated by BioID2-human Sun2. Scale bar: 10μM. (b) The NE/ER ratio is based on the men intensity detected with anti-human Sun2. 48 nuclei/condition were measured and values represent the mean + SEM.

Split BioID

In addition to improving the efficacy of BioID, a modification on the original system has also been developed as a way to detect PPIs between two known proteins of interest. More specifically, De Munter and colleagues (2017) experimented with fusing half of BirA* to one protein and the other with a different protein. They found that when these proteins are in close proximity to one another, the BirA* halves fuse to form a functional BirA*, in which subsequent biotinylation reveal both proteins as potential interactors. The only caveat of this modified BioID system is its similarity to complementation assays, such as bimolecular fluorescence complementation, which introduces tagged proteins exogenously, and therefore prevents analysis of protein dynamics in physiologically-relevant conditions. However, use of CRISPR-Cas9 system can resolve this issue by integrating the BirA*-protein of interest fusions into the genome for endogenous expression.

Conclusion

BioID has laid the foundation for novel proximity-dependent identification techniques, paving the way for further improvements and future discoveries. The ability to endogenously tag a protein of interest with BirA*, or the smaller biotin ligase employed by BioID2, via CRISPR-Cas9 systems, provides a natural

model for studying PPIs. Additionally, the ability for BioID to maintain physiologically-relevant conditions and detect weak or transient interactions, while still utilizing harsh lysis conditions to capture proteins that would otherwise be insoluble, presents a powerful advantage over other conventional PPI techniques.

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