Heredity (2014), 1–9
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www.nature.com/hdy

ORIGINAL ARTICLE

# Cuckoo search epistasis: a new method for exploring significant genetic interactions

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The advent of high-throughput sequencing technology has resulted in the ability to measure millions of single-nucleotide polymorphisms (SNPs) from thousands of individuals. Although these high-dimensional data have paved the way for better understanding of the genetic architecture of common diseases, they have also given rise to challenges in developing computational methods for learning epistatic relationships among genetic markers. We propose a new method, named cuckoo search epistasis (CSE) for identifying significant epistatic interactions in population-based association studies with a case–control design. This method combines a computationally efficient Bayesian scoring function with an evolutionary-based heuristic search algorithm, and can be efficiently applied to high-dimensional genome-wide SNP data. The experimental results from synthetic data sets show that CSE outperforms existing methods including multifactorial dimensionality reduction and Bayesian epistasis association mapping. In addition, on a real genome-wide data set related to Alzheimer's disease, CSE identified SNPs that are consistent with previously reported results, and show the utility of CSE for application to genome-wide data.

*Heredity* (2014) **0,** 000–000. doi:10.1038/hdy.2014.4

**Keywords:** single-nucleotide polymorphism; epistatic interactions; dimensionality reduction; cuckoo search

## INTRODUCTION

One source of complexity in biological systems is due to rich interactions among the components (Weng *et al.*, 1999; Hlavacek and Faeder, 2009). The advent of high-throughput genotyping and sequencing technologies has enabled the measurement of millions of single-nucleotide polymorphisms (SNPs)—the commonest type of genetic variants—in an individual. This has paved the way for understanding the genetic architecture of common diseases, but has also given rise to challenges in developing efficient methods for identifying of interactions (epistasis) among genetic variants. Several methods have been developed for analyzing genetic data that focus on epistatic interactions and include frequentist and Bayesian statistical methods and computational methods (Ritchie *et al.*, 2001; Zhang and Xu, 2005; Zhao and Xiong, 2006; Ferreira *et al.*, 2007; Yang and Liu 2007; Gayan *et al.*, 2008; Li *et al.*, 2008b; Park and Hastie, 2008; Jung *et al.*, 2009; Miller *et al.*, 2009; Wang, 2009; Wu *et al.*, 2009). A key challenge is the large number of statistical tests that have to be performed in epistasis testing especially in genome-wide association (GWA) studies that measure a large number of SNPs (Bellman and Kalaba, 1959; Steen, 2011). Moreover, high-dimensionality arising from multi-locus combinations, the relatively small sample size and the resulting data sparsity lead to lack of power in data mining methods (Cordell, 2009; Steen, 2011). To address the challenge of high-dimensionality, several feature selection methods have been applied to GWA data as a first step in identifying informative SNPs (Dube *et al.*, 2007, Saeys *et al.*, 2007).

Feature selection methods can be broadly grouped into two categories that include filter and wrapper methods (Freitas, 2002). In addition, feature selection methods that leverage biological knowledge relevant to SNPs, such as INTERSNP and Biofilter, have been developed as alternatives to filter and wrapper methods that do not sue such knowledge (Bush *et al.*, 2009; Herold *et al.*, 2009). INTERSNP is a time-efficient approach to select combinations of SNPs for a further interaction analysis based on *a priori* information obtained from either statistical evidence of single-marker association or biologic/genetic relevance information sources (Herold *et al.*, 2009). Similarly, Biofilter is a systematic knowledge-based approach to produce SNP models by integrating multiple genetic databases. This approach can be implemented with a variety of techniques such as logistic regression, classification and regression trees (Bush *et al.*, 2009).

The filter methods, typically, assess the quality of each attribute (such as a SNP) using a selection criterion. These types of feature selection methods have the advantage of being fast, but are often criticized for their inability to select relevant attributes involved in a significant interaction effect on the susceptibility of a disease or trait but that would not show sufficient individual effects for selection. Wrapper approaches, on the other hand, attempt to evaluate subsets of attributes based on sample classification accuracy. In contrast to filter methods, wrapper methods allow for all attributes to be retained and use a selection probability (Moore *et al.*, 2010). As a result, no attribute is eliminated from the analysis. There are different types of

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Received 7 May 2013; revised 9 December 2013; accepted 18 December 2013

wrapper methods and among them evolutionary computing algorithms such as the genetic algorithm (GA) and the evolution strategy methods have attracted much attention for stochastic search of Q4 epistatic interactions (Li *et al*., 2001; Ooi and Tan, 2003; Moore *et al*., 2004; Shah and Kusiak, 2004; Jirapech-Umpai and Aitken, 2005). Recently, a GA-based hybrid algorithm called genetic ensemble (GE) was proposed by Yang *et al*. (2010); this method relies on a combination of an ensemble of classifiers and a multi-objective GA (Zhang and Yang 2008; Yang *et al*., 2010).

The GE algorithm was shown to outperform other GA-based methods (Zhang and Yang, 2008). However, ensemble methods such as GE require diverse and accurate classifiers to achieve better accuracy, and identifying an efficient set of classifiers can be difficult (Dietterich, 2000). Selecting appropriate classifiers for different data sets with different number of attributes and samples, and configuring parameters properly for each classifier is challenging. Moreover, ensemble methods are likely to be more computationally expensive than methods that use a single classifier. Finally, despite the superior performance of GAs in comparison with gradient-based optimization methods, they are sensitive to parameter settings, exhibiting varying performance for different configurations of parameters such as Q5 population size, crossover frequency and mutation rate (Kumar and Chakarverty, 2011).

Motivated by evolutionary algorithms, we develop and evaluate a fast stochastic search method named cuckoo search epistasis (CSE) for identifying significant epistatic interactions in GWA studies. CSE differs from wrapper feature selection methods in that CSE does not search for a subset of informative attributes that are further analyzed for epistasis, but performs stochastic search of epistasis with no classification or training/prediction scheme. CSE used a new and relatively fast evolutionary algorithm called cuckoo search (CS), which performs better than other evolutionary algorithms. In addition, CSE uses a computationally efficient Bayesian score to evaluate combinations of SNPs for association with the phenotype.

We compare CSE's ability to identify epistatic interactions to that of multifactorial dimensionality reduction (MDR) and Bayesian epistasis association mapping (BEAM) using synthetic data sets. We also apply CSE to an Alzheimer's disease GWA data set that contains over 300 000 SNPs.

## Background

This section provides background information on Bayesian combinatorial method (BCM), which uses a Bayesian statistic for measuring genetic interactions. The CSE method uses the Bayesian statistic of BCM to evaluate combinations of SNPs. In addition, brief descriptions on MDR and BEAM are also provided.

### Bayesian combinatorial method

BCM is a search algorithm that evaluates the association between a set of interacting genetic variants and phenotype with a Bayesian statistic. It exhaustively searches over all possible combinations of SNPs and identifies combinations with a high posterior probability (Visweswaran *et al*., 2009). BCM is one of several methods that have been developed to identify epistatic variants based on a statistical measure. BCM has several advantages including the ability to handle sparse and unbalanced data, ability to deal with nonlinear interactions, and is computationally efficient, nonparametric and model free.

BCM defines an interaction model $M$, as a set of probabilities denoted by $P(Z|\mathbf{g}=(g_1,\ g_2,..,\ g_c))$ for phenotype states $Z$, given combination of genotypes $\mathbf{g}$. For a given $\mathbf{g}$ value, a multinomial distribution is assumed for $Z$ (binomial, if $Z$ has only two states). Assuming that the parameters of all multinomial distributions, that is, $\boldsymbol{\theta}_c$ *a priori* follow a Dirichlet distribution, a posterior estimate for $\boldsymbol{\theta}_c$ is obtained. The Bayes theorem is used to score the fitness of any given combinatorial model as the following:

$$P(M|Data) \propto P(Data|M)P(M) \tag{1}$$

where $P(M)$ is the prior probability of model $M$, which is assumed to have a constant value for all models and $P(Data|M)$ is the marginal likelihood, which is evaluated by the following equation:

$$P(Data|M) = \int P(Data|M,\ \boldsymbol{\theta}_c)\,P\,(\boldsymbol{\theta}_c|M)\,d\boldsymbol{\theta}_c \tag{2}$$

where $P(Data|M,\ \boldsymbol{\theta}_c)$ is the distribution of the data for a given genotype–phenotype table. Figure 1 presents an example of counts of genotypes for an interaction model with two SNPs (denoted SNP1 and SNP2) and a binary phenotype (for example, case and control).

A binomial distribution for each column (that is, the combination of genotypes for SNP1 and SNP 2) is assumed. Thus, $P(Data|M,\ \boldsymbol{\theta}_c)$ is obtained by multiplying nine independent binomial distributions.

The closed form for $P(Data|M)$ is given by the following equation and was originally derived by Cooper and Herskovits (1992): Q6

$$P(Data|M) = \prod_{i=1}^{I}\left(\frac{(\alpha_i-1)\,!}{(n_i+\alpha_i-1)\,!}\prod_{j=1}^{J}\frac{(n_{ij}+\alpha_{ij}-1)\,!}{(\alpha_{ij}-1)\,!}\right) \tag{3}$$

where $\alpha_{ij}$ are the positive hyper-parameters of a Dirichlet distribution and $\Sigma\alpha_{ij=}\alpha_i$, $I$ is the number of genotype combinations (for example, nine for a model with two SNPs with three states each), $J$ is the number of phenotype states (for example, two for a case–control data set), $n_i$ is the number of samples for a given genotype combination in an epistatic model and $n_{ij}$ is the number of samples for the $j$th phenotype and $i$th genotype combination.

Assuming that the prior distribution $P(M)$ is uniform over all possible models and the Dirichlet hyperparameters are all set to 1, the following expression gives the score that is used by BCM for an interaction model:

$$Score_{BCM}(M) = \prod_{i=1}^{I}\left(\frac{(J-1)\,!}{(n_i+J-1)\,!}\prod_{j=1}^{J}n_{ij}\,!\right). \tag{4}$$

A major limitation of BCM is that it searches exhaustively over all possible combinations of SNPs in a data set and hence it does not scale up to high-dimensional data sets. Our new algorithm overcomes

| SNP1 | AA | AA | AA | Aa | Aa | Aa | aa | aa | aa |
|---|---|---|---|---|---|---|---|---|---|
| SNP2 | BB | Bb | bb | BB | Bb | bb | BB | Bb | bb |
| Case | $n_{11}$ | $n_{21}$ | $n_{31}$ | $n_{41}$ | $n_{51}$ | $n_{61}$ | $n_{71}$ | $n_{81}$ | $n_{91}$ |
| Control | $n_{12}$ | $n_{22}$ | $n_{32}$ | $n_{42}$ | $n_{52}$ | $n_{62}$ | $n_{72}$ | $n_{82}$ | $n_{92}$ |
| Total | $\boldsymbol{n_1}$ | $\boldsymbol{n_2}$ | $\boldsymbol{n_3}$ | $\boldsymbol{n_4}$ | $\boldsymbol{n_5}$ | $\boldsymbol{n_6}$ | $\boldsymbol{n_7}$ | $\boldsymbol{n_8}$ | $\boldsymbol{n_9}$ |

**Figure 1** An example of genotype–phenotype table with two SNPs and a binary phenotype. This figure summarizes the genotype and phenotype data for a two-way interaction model with two SNPs. BCM assumes a binomial distribution for any combination of the SNP1 and SNP2 values, that is, each column of this table follows a binomial distribution. Thus, $P(Data|M,\ \boldsymbol{\theta}_c)$ is obtained by multiplying nine independent binomial distributions.

this limitation such that BCM can be applied to explore the space of possible models in high-dimensional GWA data.

### Multifactorial dimensionality reduction

MDR is a nonparametric data mining method for identifying SNP interactions (Ritchie *et al.*, 2001). MDR is based on a dimensionality reduction strategy that projects the whole genotype space to one dimension with two values, that is, low-risk or high-risk genotypes. In addition, this method does not assume a model for the data and it has the advantage of flexibility to genetic model. Since MDR was first introduced, it has been widely used in association studies. Several modifications and extensions for MDR have also been proposed either by its original authors or others (Bush *et al.*, 2007; Chung *et al.*, 2007; Gui *et al.*, 2007; Lee *et al.*, 2007; Velez *et al.*, 2007; Namkung *et al.*, 2009), which have increased its applicability. However, MDR is an exhaustive method and is mostly applicable to candidate gene studies where the number of tested SNPs is $<500$. To address this limitation, filtering methods are typically used to alleviate the computational burden and to feasible analyze genome-wide data (Cordell, 2009). The MDR software is available from http://epistasis.org.

### Bayesian epistasis association mapping

As an alternative to data mining methods, we compare CSE to a Bayesian statistical method called BEAM that has recently gained much popularity for epistasis detection. BEAM uses a model to partition markers into three categories. The first category contains markers assumed to have no impact on the disease, the second category contains markers assumed to have additive effects on the disease and the third category contains markers that are assumed to jointly influence the disease. In addition, a novel **B** statistic is proposed to exhaustively score interactions for candidate markers and is also used for further analyze of the resulting categories (Zhang and Liu, 2007). The BEAM software is available from http://www.fas.harv-ard.edu/∼junliu/BEAM.

## MATERIALS AND METHODS

This section provides details of the CSE method and then describes the experimental details including a description of the data sets. We first describe CS and a modification of CS and then describe CSE.

### Cuckoo search

CS is a metaheuristic search algorithm developed by Yang and Deb (2009, 2010). The algorithm is motivated by the reproduction strategies used by cuckoos. Cuckoos lay their eggs in the nests of other host birds and sometimes the host bird may be of a different species. The host bird may discover that the eggs are alien and either destroy them or abandon the nest. To overcome this, cuckoo eggs have evolve to mimic the eggs of the host birds. This interesting reproductive strategy of the cuckoos has been encapsulated as an optimization strategy in the form of three idealized rules:

- Each cuckoo lays one egg at a time and dumps it in a random nest. The eggs represent a set of epistatic models in our application.
- A fraction of the nests containing the best eggs are carried over to the next generation. That is, in each step of the search procedure, a fraction of the epistatic models that represents the best interaction scores are carried over.
- The number of nests is fixed and there is a probability that a host will discover an alien egg. When this happens, the host discards the nest and builds a new nest in a new location. That is, a model is discarded with probability *P* and a new model is created in its place. The probability *P* can also be interpreted as the fraction of models that is discarded.

CS uses the Lévy flight process for searching (Yang and Deb, 2009). The Lévy flight process is a random walk that consists of a series of jumps chosen from a probability density function that has a power law tail. When generating a new model in CS, a Lévy flight is performed starting at the position of a randomly selected model. If the new model is better than another randomly selected model then that model is replaced with the new model. The advantage of CS over other metaheuristic search algorithms like GA and particle swarm optimization is that there is only one parameter to adjust, namely, *P* the fraction of models that is discarded (Yang and Deb, 2009).

### Modified CS

Although CS is guaranteed to find the optimal model, the rate of convergence is not guaranteed to be fast. Walton *et al.* (2011) modified CS so that the convergence rate is increased and thus allows CS to be applied to larger model spaces.

This modified CS consists of two modifications to the original CS (Walton *et al.*, 2011). The first modification is the use of an adaptive step size in the Lévy flight. In CS, the step size is constant while in modified CS the step size decreases gradually as the algorithm progresses. This encourages more localized searching as the algorithm gets closer to the optimal model.

The second modification in modified CS is addition of information exchange among models to speed up convergence. In CS, each model is processed independently of other models while in modified CS a fraction of the best models is put into a group of top models. For each of the top models, a second model in the group is picked at random and a new model is then generated on the line connecting these two top models. Compared with CS, modified CS has two adjustable parameters: (i) the fraction of models that is discarded, and (ii) the fraction of models in the top group. Walton *et al.* (2011) determined empirically that setting the fraction of models that is discarded to 0.75 and the fraction of models that make up the top group to 0.25 yielded the best results. Figure 2 provides the pseudocode for the modified CS method.

### CSE method

The CSE method combines modified CS search with the model score used in BCM, and enables searching for significant epistatic models in data, that is, on a genome-wide scale. CSE is substantially more efficient than GE for several reasons. First, modified CS is computationally faster than other evolutionary-based methods. Second, although GE uses several classification and voting techniques, CSE uses a single but efficient scoring function. Third, the scoring function in CSE does not use cross-validation that is computationally expensive and instead uses the entire data to compute the model score. Many computational methods focus on increasing classification accuracy, which does not necessarily result in models with the largest association with the phenotype; thus, we were motivated to use an efficiently computable scoring function instead of a classification technique. Figure 3 provides an overview of the main steps in CSE.

In CSE, each egg represents an interaction model, which is a combination of different SNP markers to be evaluated for their association with the disease of interest. Dependent on user-defined SNP interaction order, for example, a *k*-way interaction detection setting, to generate the initial set of models we assume each egg to represents a vector with *k* components. Then, CSE assigns a random value in [0, 1], based on random walks, for each component of the egg, which represents a SNP. It is guaranteed that each egg cannot contain the same SNP markers. Next, a model score is calculated for each egg using the BCM score. CSE tries to allocate continuous numbers to each component of the eggs in order to get nearer to the interaction model with a higher model score. This procedure can be repeated for detecting any-way SNP interaction model.

In GWA data where an astronomical number of potential interaction models exist, the data redundancy and correlation structure between SNPs often produce inconsistent results. To address this problem, we considered several modifications to CSE so that it can be applied to GWA data. In the first stage, CSE partitions the SNPs into *m* groups with $\mathrm{L_i}$ SNPs, $\mathrm{i} = 1,..\mathrm{m}$, $\sum_{\mathrm{i}=1}^{\mathrm{m}} \mathrm{L_i} = \mathrm{L}$. This partitioning of SNPs can be with respect to either their natural position in the genome or their associated gene. This is because there is considerable correlation among neighboring SNPs in the genome as measure by linkage disequilibrium (LD). Considering the *k*–SNP interaction detection problem, *k* groups are selected out of all *m* groups. Then, to construct each egg of the generation, a vector of *k* dimensions with continuous numbers in [0, 1] is

```
$A \leftarrow MaxLévyStepSize$
$\varphi \leftarrow GoldenRatio$
Initialize a population of $n$ host nests $x_t$ $(t = 1,2,..,n)$
for all $x_t$ do
    Calculate fitness $F_t = f(x_t)$
end for
Generation Number $G \leftarrow 1$
While Number Objective Evaluations < Number of generations do
    $G \leftarrow G + 1$
    Sort nests by order of fitness
    for all nests to be abandoned do
        Current position $x_i$
        Calculate Lévy flight step size $\alpha \leftarrow A/\sqrt{G}$
        Perform Lévy flight from $x_i$ to generate new egg $x_k$
        $x_i \leftarrow x_k$
        $F_i \leftarrow f(x_i)$
    end for
    for all of the top nests do
        Current position $x_i$
        Pick another nest from the top nests at random $x_j$
        if $x_i = x_j$ then
            Calculate Lévy flight step size $\alpha \leftarrow A/G^2$
            Perform Lévy flight from $x_i$ to generate new
            egg $x_k$
            $F_k = f(x_k)$
            Choose a random nest $l$ from all nests
            if ($F_k > F_l$) do
                $x_l \leftarrow x_k$,    $F_l \leftarrow F_k$
            end if
            else
                $dx = |x_i - x_j|/\varphi$
                Move distance $dx$ from the worst nest to the
                best nest to find $x_k$
                $F_k = f(x_k)$
                Choose a random nest $l$ from all nests
                if ($F_k > F_l$) then
                    $x_l \leftarrow x_k$,    $F_l \leftarrow F_k$
                end if
            end if
        end for
end while
```

**Figure 2** Pseudocode for modified CS.

assigned by the CSE algorithm as described earlier such that each component of the vector represents a SNP that is selected from one of the $k$ selected groups. In the next steps of the algorithm, the top 100 highest scored interaction models with $k$ SNPs for the selected groups are identified. The procedure continues by selecting other $k$ groups either until a predefined number of iteration is reached or all possible $k$ groups have been examined exhaustively. Finally, the best interaction models from the resulting models are reported. Figure 4 gives the pseudocode for CSE.

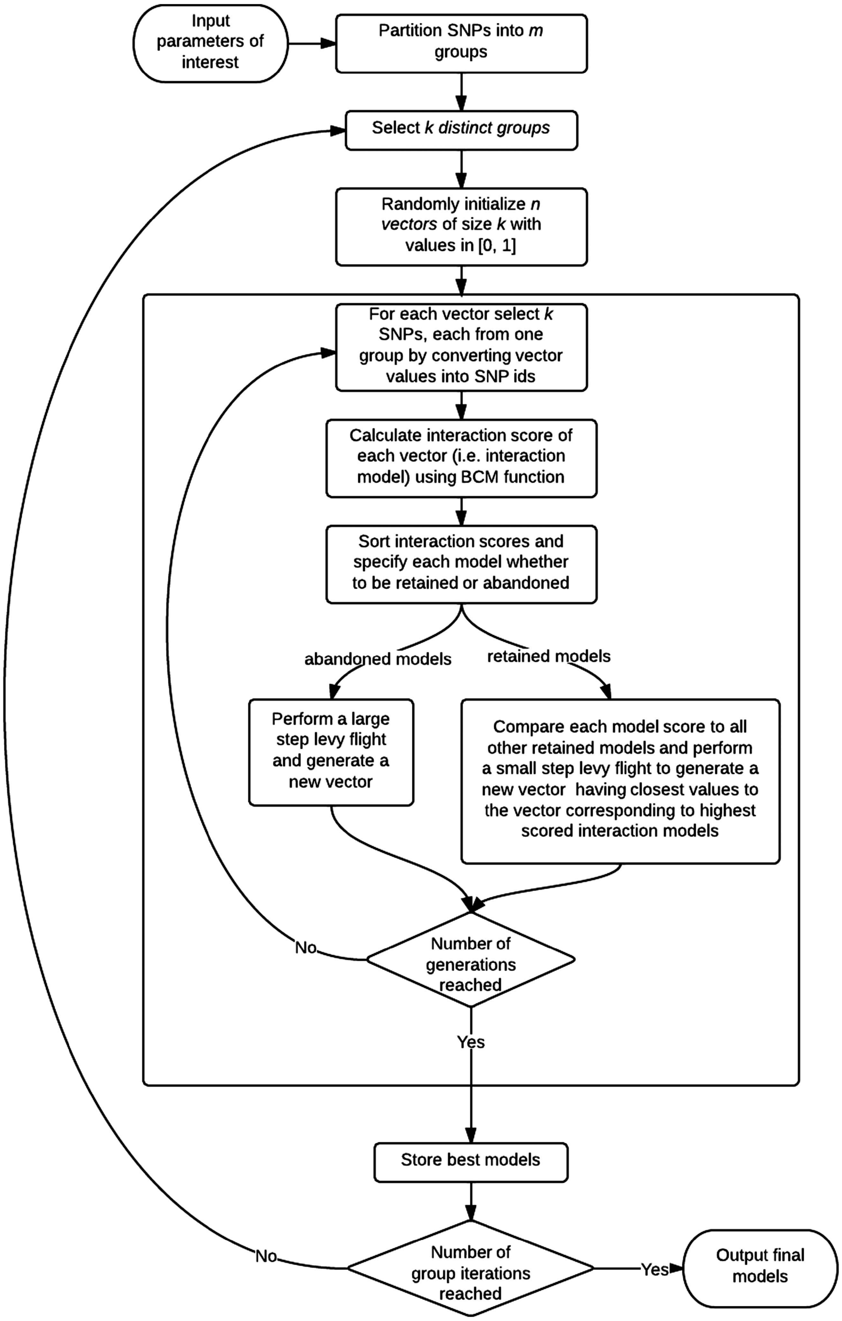

Input parameters of interest
Partition SNPs into *m* groups
Select *k distinct groups*
Randomly initialize *n vectors* of size *k* with values in [0, 1]
For each vector select *k* SNPs, each from one group by converting vector values into SNP ids
Calculate interaction score of each vector (i.e. interaction model) using BCM function
Sort interaction scores and specify each model whether to be retained or abandoned
abandoned models
retained models
Perform a large step levy flight and generate a new vector
Compare each model score to all other retained models and perform a small step levy flight to generate a new vector having closest values to the vector corresponding to highest scored interaction models
No
Number of generations reached
Yes
Store best models
No
Number of group iterations reached
Yes
Output final models

**Figure 3** Flowchart showing the main steps in CSE.

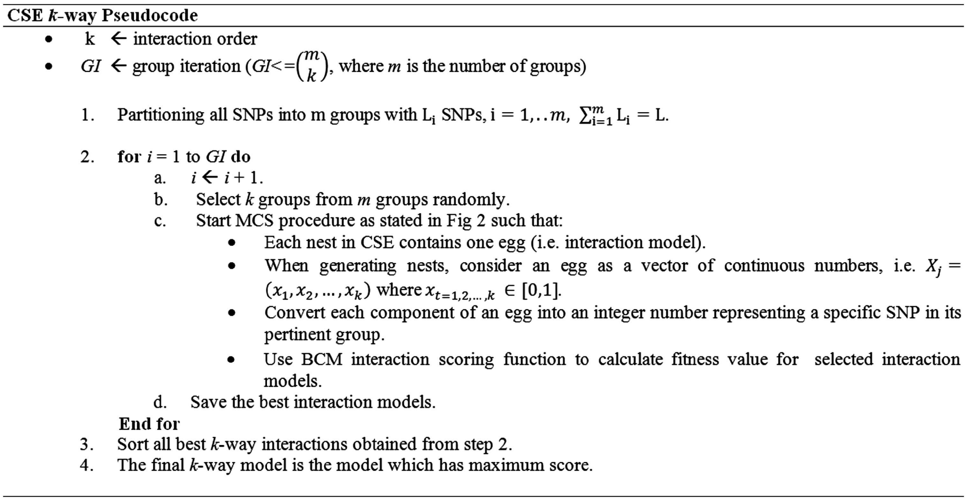
## Grouping of SNPs

We partitioned GWA data into groups of SNPs in the first stage. Two partitioning schemes that are likely to be useful are:

(a) Group SNPs according to their natural genomic order such that adjacent SNPs are in the same group.
(b) Group SNPs according to their associated genes such that SNPS on a gene are in a single group.

There are other ways to partition SNPs in groups that may be useful. For instance, knowledge obtained from gene ontology or gene expression experiments may be used to partition SNPs in an association study. Determining which partitioning scheme is to be chosen depends on the goals of the analyses and the computational costs. If the goal is to detect gene–gene interactions as well as SNP–SNP interactions, then grouping SNPs based on genes is the preferred method. If the goal is to identify only SNP–SNP interactions, then partitioning SNPs according to their natural genomic order or based on their LD may be the preferred method. From the computational perspective, partitioning based on LD is more expensive than partitioning that is based on the genomic order or based on gene membership.

In addition, a partitioning scheme should balance the group size (that is, number of SNPs per group) and the number of groups. Considering dimension of data and computational facilities, the group size and number of groups can be defined by the user based on the importance of LD in the resulted interaction models. As a big group size induces less number of groups, which include SNPs with low LDs between two groups; the chance of deriving mixed results because of LD is expected to be minimized. However, as CSE, in a genome-wide scale, does not consider searching for interaction models inside



```
CSE k-way Pseudocode
•  k ← interaction order
•  GI ← group iteration ($GI <= \binom{m}{k}$, where m is the number of groups)

1.  Partitioning all SNPs into m groups with $L_i$ SNPs, $i = 1,..m$, $\sum_{i=1}^{m} L_i = L$.

2.  for i = 1 to GI do
        a.  $i \leftarrow i + 1$.
        b.  Select k groups from m groups randomly.
        c.  Start MCS procedure as stated in Fig 2 such that:
                •  Each nest in CSE contains one egg (i.e. interaction model).
                •  When generating nests, consider an egg as a vector of continuous numbers, i.e. $X_j = (x_1, x_2, \ldots, x_k)$ where $x_{t=1,2,\ldots,k} \in [0,1]$.
                •  Convert each component of an egg into an integer number representing a specific SNP in its pertinent group.
                •  Use BCM interaction scoring function to calculate fitness value for selected interaction models.
        d.  Save the best interaction models.
    End for
3.  Sort all best k-way interactions obtained from step 2.
4.  The final k-way model is the model which has maximum score.
```

**Figure 4** Pseudocode for CSE method.

a group; it is likely to ignore evaluating a possibly high-scored interaction model in the first place. As our main focus in this study was developing the main procedure of detecting interaction effects rather than proposing a specific procedure to partition SNPs, we have selected these parameters based on limited analyses of simulated data. However, in order to comprehensively consider the effect of these parameters, sensitivity analysis should be performed before implementation of the procedure.

### Experimental methods

We evaluated the performance of CSE using several synthetic data sets. For comparison, we used two control methods including MDR and BEAM that represent two different schools of epistasis methods. We compared the performance of CSE, MDR and BEAM in terms of power and computational time.

### Synthetic data

We used two different synthetic data sources that have been developed previously. Detection of two loci epistasis was assessed using the Velez data (Velez *et al.*, 2007), and higher order SNP interactions detection was assessed using the Himmelstein data (Himmelstein *et al.*, 2011).

The Velez data set includes 20 different non-linear genetic models (Velez *et al.*, 2007). These data sets were developed with a case–control ratio of 1:1 and for penetrance functions with variable heritability levels (0.01 and 0.4) and varying minor allele frequencies (MAFs; 0.2 and 0.4). Genotype frequencies for epistasis models were consistent with Hardy–Weinberg proportions. The evaluations were performed on sample sizes of 400, 800 and 1600 with 1000 SNPs per individual. Each genetic model included 100 data sets in which two functional SNP markers were embedded within a set of 998 non-interacting SNP markers with a weak marginal effect. A total number of 2000 data sets were used for each sample size. These data sets are available online from http://discovery.dartmouth.edu/epistatic_data/.

We also used Himmelstein data sets with three to five functional SNPs, which had been generated with no predefined genetic models, to evaluate methods in identifying higher order interactions. For any interaction order, the data folders consisted of 100 data sets each having 1500 cases and 1500 controls for a SNP number as high as the considered interaction order. Assuming Hardy-Weinberg equilibrium proportions and MAF of 0.5, we randomly generated additional SNP data to embed with the Himmenstein data using a multinomial distribution. After embedding Himmelstein data with our generated data sets, the resulting data sets for any interaction order contained 1000 SNPs for 3000 samples. These data sets are available online from http://discovery.dartmouth.edu/model_free_data/.

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### GWA data

To evaluate the performance of the CSE method on a real GWA data set, we used a late-onset Alzheimer's disease (LOAD) GWA data set that was collected and analyzed previously (Reiman *et al.*, 2007). Genotype data were obtained from 1411 samples including 861 cases diagnosed with LOAD and 550 controls. Of the 1411 samples, the case–control status was defined as neuropathological LOAD determined from brain tissue in 1047 samples and was determined based on clinical diagnosis for 364 samples. The genotype data consist of 502 627 SNPs that were measured using the Affymetrix chip, from which 312 316 SNPs remained after applying quality controls by the original investigators. In addition, the original investigators measured two apoplipoprotein E genotypes (rs429358 and rs7412) by either pyrosequencing or restriction fragment length polymorphism analysis because they are not measured on the Affymetrix chip. The apoplipoprotein E is the most reliably replicated genetic variant associated with LOAD (Corder *et al.*, 1993; Pappassotiropoulos *et al.*, 2006; Coon *et al.*, 2007). On the basis of the two genotypes, the apoplipoprotein E gene has three common variants e2, e3 and e4 where e2 is the low-risk allele, and each copy of the e4 allele increases the risk.

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## RESULTS

We first present results from the synthetic data sets and then present results from the LOAD GWA data set.

### Application to synthetic data

We have performed an extensive analysis of synthetic data to assess the performance of the CSE method and compare its performance with that of MDR and BEAM methods. We used the ReliefF filter method, which is implemented in the MDR software package, to filter the top 100 informative SNPs out of 1000 SNPs before testing for interaction detection. Thereafter, the MDR procedure was implemented to detect the best interaction models of different interaction orders. The analysis parameters such as number of chains/burn-ins and configurations of grouping priors for the BEAM algorithm were set according to recommended parameter settings by the authors (included in BEAM software package). We estimated the power as the proportion of the 100 replicate data sets for which the algorithm ranks the two functional SNPs as the top two SNPs.

Figure 5 presents the power comparison for the three methods for 20 different penetrance functions and for three sample sizes based on the Velez data set for two interacting loci. As can be seen in Figure 5, the performance of all methods is positively correlated with sample size. MDR had better performance than BEAM for genetic models with large values of MAF and heritability (models in the top two rows in Figure 5). However, for smaller values of MAF and heritability (models in the bottom two rows in Figure 5) BEAM performed better than MDR. On the Himmelstein data sets (see Table 1), BEAM does better than MDR in two out of three experiments. These results are supportive of BEAM having better performance than MDR in situations where interacting loci have modest main effects and interaction effects, and similar results were obtained in a comprehensive study that evaluated several epistasis detection methods (Chen *et al*., 2011).

CSE performed better than MDR and BEAM on both the Velez and Himmelstein data sets with marked increase in power for models with low heritability. The failure of the preprocessing filter procedure to retain the functional SNPs at the first step likely explains the weak performance of MDR.

We compared the computational time of the three methods using a desktop computer of 2.26 GHz CPU and 4 GB RAM. Table 2 summarizes the average computational time to run each data set. For a comprehensive comparison, we estimated the average running time for MDR when implemented exhaustively. As it is shown in Table 2, the running time of CSE is nearly eight times less than exhaustive MDR and two times less than BEAM.

### Application to GWA data

We imputed the missing genotypes in the GWA data using the IMPUTE software (Howie *et al*., 2009). A genotypic test using $\chi^2$ statistic with 2 df, was applied to each of the SNPs using the PLINK

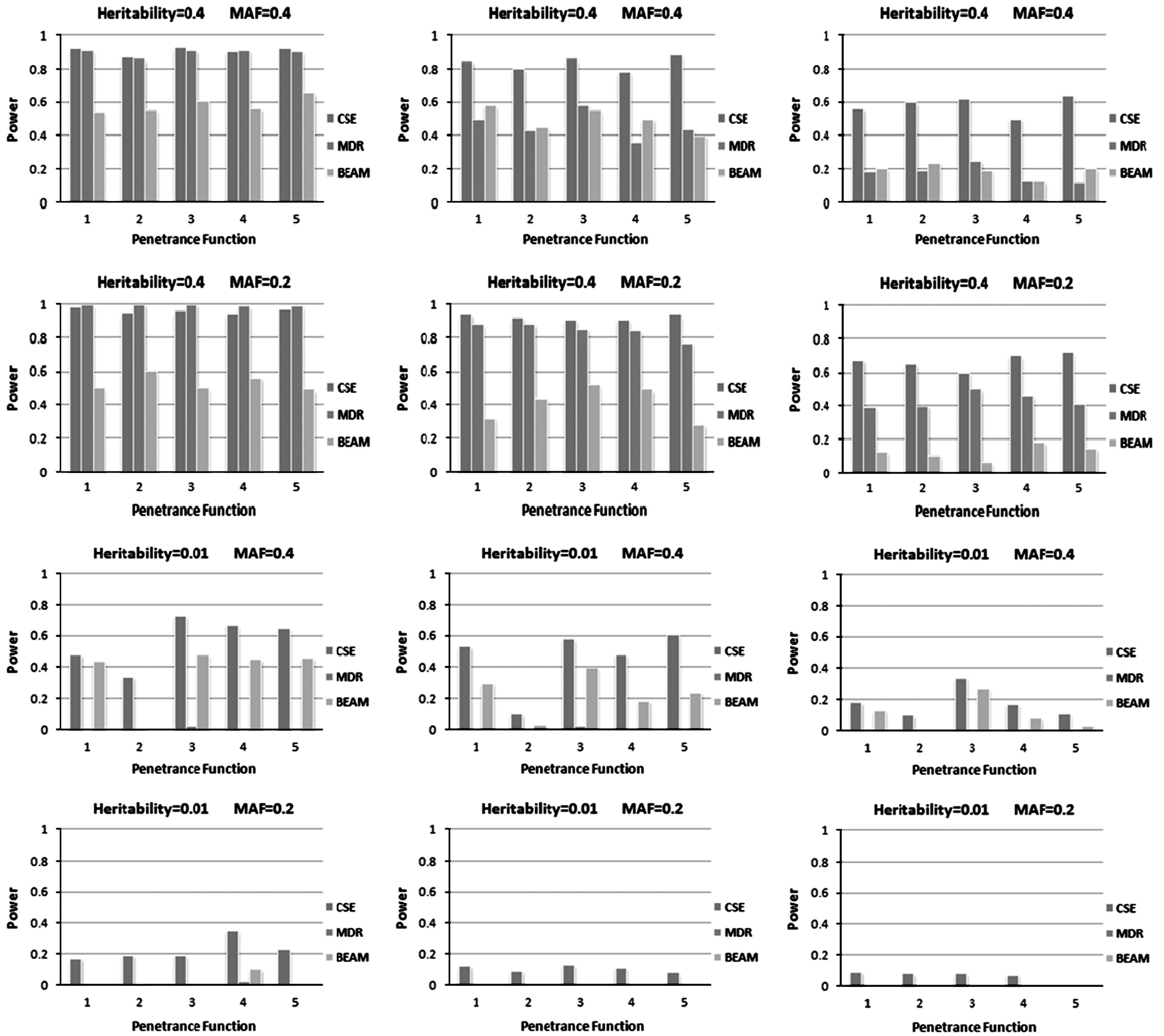

**Figure 5** Powers obtained by three epistasis detection methods (CSE, MDR and BEAM) on synthetic data containing two interacting SNPs. The figure gives the power of the methods for three sample sizes of 1600, 800 and 400 with equal numbers of cases and controls. For each sample size, 20 penetrance functions with two MAFs and two heritability levels are examined. For each penetrance model, 100 data sets with 1000 SNPs are examined. The highest scoring interactions were evaluated using CSE, MDR and BEAM for each data set. Finally, the power was estimated as the number of correctly detected interacting SNPs divided by the number of SNPs in the data set.

**Table 1 Powers obtained by three epistasis detection methods for three-way, four-way and five-way interactions**

| *Interaction order* | *CSE* | *MDR* | *BEAM* |
|---|---|---|---|
| Three-way | 69 | 45 | 48 |
| Four-way | 47 | 40 | 35 |
| Five-way | 18 | 12 | 31 |

Abbreviations: BEAM, Bayesian epistasis association mapping; CSE, cuckoo search epistasis; MDR, multifactorial dimensionality reduction; SNP, single-nucleotide polymorphism.
Power is estimated as the proportion of the 100 replicate data sets for which the method ranks the functional SNPs as the top three, four or five SNPs.

**Table 2 Running times for the three epistasis detection methods**

| *Sample size* | *CSE* | *MDR (exhaustive)* | *MDR (filtered)* | *BEAM* |
|---|---|---|---|---|
| 1600 | 69 s | 480 s | 16 s | 134 s |
| 800 | 54 s | 390 s | 12 s | 102 s |
| 400 | 40 s | 190 s | 7 s | 75 s |

Abbreviations: BEAM, Bayesian epistasis association mapping; CSE, cuckoo search epistasis; MDR, multifactorial dimensionality reduction; SNP, single-nucleotide polymorphism.
The running times were obtained from a data set with 1000 SNPs and different sample sizes of 400, 800 and 1600.

**Table 3 List of top-ranked SNPs that interact with the APOE SNP rs7412 that were identified by CSE**

| *Rank* | *SNP identifier* | *Chromosome number* | *Associated gene* | *Interaction score* |
|---|---|---|---|---|
| 1 | rs7079348 | 10 | *C10ORF11* | –833.573 |
| 2 | rs934745* | 18 | *MAPK4* | –838.546 |
| 3 | rs10499687* | 7 | *VWC2* | –840.237 |
| 4 | rs2517509 | 6 | *MUC21* | –841.314 |
| 5 | rs2122339 | 2 | *STIM2* | –841.828 |
| 6 | rs7817227 | 8 | *C8orf80* | –843.664 |
| 7 | rs2779556* | 9 | *GABBR2* | –845.716 |
| 8 | rs475093 | 1 | *LOC440585* | –846.129 |
| 9 | rs17126808 | 8 | *PSD3* | –849.232 |
| 10 | rs7585710 | 2 | *ATP6V1C2* | –850.014 |
| 11 | rs7097398 | 10 | *KIF20B* | –854.823 |
| 12 | rs12162084 | 16 | *HS3ST4* | –856.846 |
| 13 | rs473367 | 8 | *POU5F1* | –857.432 |
| 14 | rs4394475 | 9 | *NXNL2* | –857.973 |
| 15 | rs17330779 | 7 | *NRCAM* | –857.989 |
| 16 | rs17048904 | 4 | *NDST4* | –858.782 |
| 17 | rs17151710 | 5 | *CSNK1G3* | –858.302 |
| 18 | rs1763351 | 1 | *COL11A1* | –859.012 |
| 19 | rs10824310 | 1 | *PRKG1* | –859.285 |

Abbreviations: APOE, apoplipoprotein E; CSE, cuckoo search epistasis; SNP, single-nucleotide polymorphism.
Of the 19 SNPs in the table, 16 SNPs have been initially identified in both Reiman *et al.* (2007) and Li *et al.* (2008a). Three of the 19 SNPs (indicated by a star) have not been documented in previous studies and are potential candidates for future studies. The interaction score is the natural logarithm of the Bayesian score given in Equation 4.

software (Bender *et al.*, 2007). Finally, 76 755 SNPs with *P*-value $<0.2$ were retained for further analysis for epistatic interactions.

In order to detect two-way interactions, we partitioned the SNPs into 295 groups each containing 260 SNPs considering a balance between group size and number of groups. Then, CSE was applied to explore every pairwise partition for epistatic interactions. We set CSE to identify the 100 top-ranked epistatic interactions for any experiment on a pair of partitions. Our experimental results with the LOAD data set identified the apoplipoprotein E SNP rs7412 to have the strongest association with LOAD. This is consistent with the knowledge that the SNP is the most representative disease involved in high-ranked interaction models with LOAD (Combarros *et al.*, 2009). Q10

Table 3 summarizes the 19 top-ranked SNP interactions in the LOAD data set detected by CSE. Of these SNPs, 16 have been initially identified as LOAD-associated SNPs in other GWA studies (Reiman *et al.*, 2007; Li *et al.*, 2008a; Shi *et al.*, 2010). Q11

## DISCUSSION

The high dimensionality of SNP data sets poses a challenge to exhaustive search methods for the detection of genetic interaction in genome-wide data sets. Traditional filtering methods to reduce the number of SNPs rely on the selection of SNPs based on marginal effects and have limited ability to detect interactions in the absence of marginal effects. The newer multivariate filter methods such as ReliefF, which we used in conjunction with the MDR method, can detect interactions in the absence of marginal effects. However, they may suffer from lack of power. Our experimental results highlighted in particular the loss of power for genetic models with low heritability. Evolutionary computing methods, such as advanced wrapper methods, have gained more popularity in recent years as an alternative, however, they have classification limitations mostly because of the capacity of features, which does not necessarily identify epistatic interactions.

In this paper, we proposed CSE as a new method, which combines a computationally efficient Bayesian scoring function with an evolutionary-based heuristic search algorithm, for epistasis detection in genome-wide data. Although relying on an evolutionary computing strategy, CSE is much faster than other algorithms of its kind. A differentiating characteristic of CSE is that in contrast to computational methods such as wrapper methods, which rely on data classification, CSE uses an efficient function to score epistatic interactions. The epistatic scoring function gains in efficiency by eliminating the need to perform cross-validation. Furthermore, CSE can efficiently test for a variety of different multi-locus epistatic models, and because it does not eliminate SNP markers with filtering, it can conduct a heuristic search among all possible interaction models for all available SNPs in the data set.

The results obtained with synthetic data support the additional value of CSE over other epistasis methods for the detection of epistatic interaction. The results illustrate that preprocessing procedures may reduce the computational burden at the cost of power. The application of CSE to a real GWA data set of LOAD provided results that are consistent with previously reported findings, highlighting the value of CSE for the exploration of epistatic interactions in genome-wide data.

### Software availability

The software package that implements CSE, the documentation and illustrative examples are available from the following website: http://lbb.ut.ac.ir/Download/LBBsoft/CSE.

## DATA ARCHIVING

There were no data to deposit.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We thank Jason H Moore, PhD and Peter C Andrews, AB for helpful discussions and assistance with the MDR algorithm and software. We also thank Zahra Narimani for her technical assistance.

*Author contributions:* MA, AM-N, AG and SV designed the study; MA and HS developed the algorithm; MA, HS and SV analyzed the data, and all authors wrote and edited the paper.

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