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REVIEW

Natural epigenetic variation in bats and its role in evolution

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ABSTRACT

When facing the challenges of environmental change, such as habitat fragmentation, organisms have to adjust their phenotype to adapt to various environmental stresses. Recent studies show that epigenetic modifications could mediate environmentally induced phenotypic variation, and this epigenetic variance could be inherited by future generations, indicating that epigenetic processes have potential evolutionary effects. Bats living in diverse environments show geographic variations in phenotype, and the females usually have natal philopatry, presenting an opportunity to explore how environments shape epigenetic marks on the genome and the evolutionary potential of epigenetic variance in bat populations for adaptation. We have explored the natural epigenetic diversity and structure of female populations of the great roundleaf bat (*Hipposideros armiger*), the least horseshoe bat (*Rhinolophus pusillus*) and the eastern bent-winged bat (*Miniopterus fuliginosus*) using a methylation-sensitive amplified polymorphism technique. We have also estimated the effects of genetic variance and ecological variables on epigenetic diversification. All three bat species have a low level of genomic DNA methylation and extensive epigenetic diversity that exceeds the corresponding genetic variance. DNA sequence divergence, epigenetic drift and environmental variables contribute to the epigenetic diversities of each species. Environment-induced epigenetic variation may be inherited as a result of both mitosis and meiosis, and their potential roles in evolution for bat populations are also discussed in this review.

KEY WORDS: DNA methylation, Chiroptera, Mammals, Environmental adaptation

Introduction

When facing challenges from environmental changes or stresses, organisms have to adapt to the changed environment by genetic mechanisms, physiological adaptability, phenotypic plasticity or moving to a new, more suitable area. The genetic changes may support abundant phenotypic variation in organisms for environmental adaptation, but they occur slowly and cannot keep pace with the rapidly changing environment (Bonduriansky et al., 2012). Recent studies have suggested that epigenetic modifications in eukaryotes could affect genetic expression and thus may mediate phenotypical variation in response to rapid and unpredictable environmental changes without genetic divergence (Dolinoy et al., 2007; Gao et al., 2010; Kucharski et al., 2008). These environmentally induced epigenetic patterns may even be stably inherited by future generations (Grant-Downton and Dickinson, 2006; Jablonka and Lamb, 1998; Richards, 2006), which provides an additional pathway for environmental adaptation and produces a challenge to the Modern Evolutionary Synthesis (Bossdorf et al., 2008; Jablonka and Lamb, 1995; Jablonka et al., 2005), in that the epigenetic process is another source of random variation in natural populations in addition to genetic variance (Massicotte et al., 2011; Schmitz et al., 2011).

DNA methylation, one of the key epigenetic markers, is a covalent modification that occurs at the fifth carbon position of a cytosine ring by DNA methyltransferases. DNA methylation can affect the transcription of genes by impeding the binding of transcriptional proteins to the gene or recruiting additional proteins to the locus in methyl-CpG binding domain protein forms. In mammals, DNA methylation plays an important role in gene regulation, imprinting and X-chromosome inactivation and is also associated with immunity, disease and ageing. Studies conducted with animals and plants have suggested that environmental change, such as diets and behaviours, could induce DNA methylation variance of organisms or their offspring (Crews et al., 2007; Maleszka, 2008; Waterland and Jirtle, 2003). For example, in viable yellow agouti (A^{vy}) mice, maternal exposure to the environmental toxicant bisphenol A causes a decrease in methylation at nine CpG sites in an intracisternal A particle retrotransposon upstream of the *Agouti* gene, and thus alters the coat colour distribution of their offspring (Dolinoy et al., 2007). Differences in diet determine whether honeybee larvae develop into either sterile workers or reproductive queens by altering their genomic DNA methylation (Kucharski et al., 2008). The different degrees of rat maternal pup-licking and grooming and arched-back nursing could alter the DNA methylation at a glucocorticoid receptor gene promoter in the hippocampus of their offspring, and thus cause them to exhibit individual differences in response to the surrounding environment (Weaver et al., 2004). Nätt and colleagues (Nätt et al., 2012) found that domestication processes could affect the levels of methylation and expression in selective sweep regions in domesticated chicken and red junglefowl (their ancestor). These studies suggest that variation in DNA methylation may increase the adaptation potential of organisms under environmental stress as an additional system in evolution (Bossdorf et al., 2008; Jablonka and Lamb, 1995; Jablonka et al., 2005).

Most of the above studies are based on model systems that have been altered by human selection (Shorter et al., 2012; Vrana, 2007). Although they could establish the correlation between epigenetic and phenotypic variation effectively, they may not reflect the epigenetic features in the real world. The interaction between epigenetic processes and natural environments may be more complex, and epigenetic processes may take part in advanced physiological processes such as botanic hybridization and polyploidization (Ellstrand and Schierenbeck, 2000; Liu and Wendel, 2003; Rapp and Wendel, 2005; Salmon et al., 2005), and ecological niche differentiation (Herrera et al., 2012; Schrey and Richards, 2012), and thus may even cause speciation (Boffelli and Martin, 2012; Flatscher et al., 2012). Thus, it is important to investigate epigenetic variation and epigenetic inheritance and estimate the potential role of epigenetic variation in producing new phenotypes and responding to global environmental change in natural populations (Bossdorf et al., 2008; Ledón-Rettig, 2013).

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Variations in natural genomic methylation have been investigated in populations of several plant species. The wind-pollinated tree species *Betula ermanii* lives in contrasting habitats in the Changbai Mountains of northeastern China – subalpine forest and alpine tundra regions – and shows significant genetic and epigenetic variation, suggesting that genome-wide DNA methylation variance may be a source of random variation in facilitating the environmental adaptation of *B. ermanii* (Wu et al., 2013). Populations of the alligator weed *Alternanthera philoxeroides*, which grows in both terrestrial and aquatic habitats and appears to have little genetic variance but extensive morphological differences, shows genome-wide epigenetic reprogramming and similar phenotypes in response to different common-garden environments, indicating the flexibility and the role of the epigenetic system in mediating environmentally induced phenotypic variation (Gao et al., 2010). Populations of the white mangrove *Laguncularia racemosa* live in the salt marsh and riverside with little genetic variation but have significant phenotypic and DNA methylation differentiation (Lira-Medeiros et al., 2010). With limited genetic divergence, the invasive *Fallopia* species complex – which lives in different areas, including marsh, beach and roadside – exhibits an extensive epigenetic diversity, suggesting that epigenetic marks differentiate faster than genetic ones and could contribute to phenotypic differentiation in response to diverse environments (Richards et al., 2012).

Studies on the natural epigenetics of wild animal populations in terms of environmental adaptation and evolution are scarce. In the salmonid *Oncorhynchus mykiss*, fish raised in hatcheries have fewer surviving offspring than wild fish; Blouin et al. (Blouin et al., 2010) attempted to explain the phenomenon in terms of epigenetic variation, but no significant difference in epigenetic patterns between the two populations was found. However, this work is the first to investigate the natural population epigenetic variation in animals. A similar study showed that salt-enriched diets could induce short-term, reversible genome-wide methylation changes in hatchery-reared trout (*Salmo trutta*) when they migrate to the sea from freshwater (Morán et al., 2013). Studies conducted in the clonal fish *Chrosomus eos-neogaeus* (Massicotte et al., 2011) and the house sparrow, *Passer domesticus* (Schrey et al., 2012), showed specific population epigenetic patterns, which suggested that natural epigenetic variation facilitates the environmental adaptation of these animals.

Bats, animals of the order Chiroptera, are the only mammals that have evolved true flight. The capacity of flight has enabled bats to be one of the most widely distributed groups of mammals. They are important components of biodiversity and play critical ecological roles in insect control, plant pollination and seed dissemination (Kunz and Fenton, 2003). Changes in their populations greatly affect ecological balance and thus they reflect wider scale impacts on the biota of interest. In addition, bats are considered as bioindicators of environmental change because of their sensitivity to environmental variation (Jones et al., 2009); environmental changes, such as habitat destruction, environmental pollution and global climate change significantly affect the diversity, population dynamics, development and behaviour of bats (Jones et al., 2009; Mann et al., 2002). For instance, light pollution from illumination of buildings could cause stunted growth of house-dwelling bats (Boldogh et al., 2007) and even non-tactile stimuli could arouse hibernating bats (Thomas, 1995). Populations of bats in various habitats also show geographic variation in phenotype, such as morphology and acoustic traits (Maharadatunkamsi et al., 2000; Sun et al., 2013). Thus, bats may present an opportunity to explore the evolutionary potential of epigenetic variance in environmental adaptation. In this review, we investigate three aspects of how epigenetic variation facilitates bat populations to adapt to diverse environments: (1) the extent and structure of DNA methylation within and among bat populations; (2) the influencing factors that contribute to the extensive epigenetic diversity of bat populations; and (3) the potential function of DNA methylation in evolution for bats.

Fig. 1. The bat species studied. (A) *Rhinolophus pusillus*, (B) *Hipposideros armiger* and (C) *Miniopterus fuliginosus*.

Natural population epigenetics in bat populations

In order to investigate the common natural epigenetic characteristics in bat populations, three diverse species with disparate features in their morphologies, calls and behaviours were selected, including the great roundleaf bat, *Hipposideros armiger* (Hodgson 1835), the least horseshoe bat, *Rhinolophus pusillus* Temminck 1834, and the bent-winged bat, *Miniopterus fuliginosus* Hodgson 1835 (Fig. 1) (Liu et al., 2012; Norberg and Rayner, 1987). Female individuals (adult) of the three bat species were selected because most of them have strong natal philopatry (the behaviour of remaining in, or returning to, an individual's birthplace), which offers the opportunity to estimate the effects of local ecological factors on their epigenetic diversity. In addition, bats in hibernation were excluded from this study because DNA methylation is likely to be different between the periods of hibernation and normal status in bats (Morin and Storey, 2009).

Nine populations of *H. armiger* comprising 45 females, nine populations of *R. pusillus* comprising 39 females and eight populations of *M. fuliginosus* comprising 47 females in total were selected to explore the natural epigenetic characteristics in bats (Table 1, Fig. 2). All samples were collected during September–October in 2009 and 2010, and deposited in the Museum of Natural History of Northeast Normal University, Jilin Province, China. Genomic DNA was extracted from muscle tissue using a UNIQ-10 Column Animal Genome DNA Extraction Kit (SK1205, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd).

Methods to detect population epigenetics

The methylation-sensitive amplified polymorphism protocol (MSAP) is the most commonly used technique to detect whole-genome epigenetic variation among individuals and populations (Schrey et al., 2013). Modified from the amplified fragment length polymorphism (AFLP) technique (Reyna-López et al., 1997), MSAP uses *Eco*RI (rare cutter) with either one of two methylation-sensitive isoschizomer restriction enzymes, *Hpa*II and *Msp*I, which recognize the same

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Table 1. Location of sample populations and individual numbers of the three bat species

Locations	Abbreviation for location	Longitude (°E)	Latitude (°N)	No. of *H. armiger*	No. of *R. pusillus*	No. of *M. fuliginosus*
Nanzhao, Henan Province	HeNNZ	33.50	–	–	–	5
Tongbai, Henan Province	HeNTB	32.81	113.40	–	5	–
Wanyuan, Sichuan Province	SCWY	32.12	107.95	6	–	–
Hanshan, Anhui Province	AHHS	31.62	118.13	–	6	–
Yixing, Anhui Province	JSYX	31.25	119.78	–	3	–
Wuhu, Anhui Province	AHWH	31.05	118.30	–	5	4
Huzhou, Zhejiang Province	ZJHZ	30.95	120.09	–	3	–
Wuyuan, Jiangxi Province	JXWY	29.39	117.70	–	–	6
Chongqing	CQ	29.28	107.71	–	3	–
Qianshan, Jiangxi Province	JXQS	28.21	117.69	5	–	5
Suiyang, Guizhou Province	GZSY	27.99	107.18	5	–	–
Lengshuijiang, Hunan Province	HuNLSJ	27.75	111.57	–	–	4
Jianggangshan, Jiangxi Province	JXJGS	26.60	114.20	6	–	–
Xuanwei, Yunnan Province	YNXW	26.22	103.81	–	–	8
Pingtan, Fujian Province	FJPT	25.43	109.74	–	6	–
Guilin, Guangxi Province	GXGL	25.39	110.68	–	5	–
Anlong, Guizhou Province	GZAL	25.28	105.54	5	–	–
Shaoguan, Guangdong Province	GDSG	24.77	113.56	4	–	–
Baise, Guangxi Province	GXBS	24.34	106.58	6	–	–
Yuxi, Yunan Province	YNYX	23.55	102.27	4	3	8
Simao, Yunnan Province	YNSM	22.60	100.71	4	–	–
Haikou, Hainan Province	HaiNHK	19.94	110.21	–	–	7

restriction site (5′-CCGG-3′) but have different cytosine methylation sensitivities (Vos et al., 1995). *Hpa*II is sensitive to full methylation of either C but cleaves the hemi-methylated external C, whereas *Msp*I cleaves fully-methylated internal C, while both isoschizomers can cleave non-methylated CCGG sites. Thus, the technique can be used reflect the difference in genome-wide methylation patterns between individuals or populations.

However, the disadvantage of MSAP is that it can only detect methylation on 5′-CCGG-3′ and screen anonymous loci that may not be linked to the traits. Some other potential disadvantages have been reviewed elsewhere (Schrey et al., 2013). Techniques like bisulphite sequencing, microarrays or next-generation sequencing may overcome the shortcomings of the MSAP technique. However, the MSAP technique is well suited for non-model species, and is applied widely to investigate natural epigenetic variance in wild populations (Blouin et al., 2010; Herrera and Bazaga, 2011; Lira-Medeiros et al., 2010; Richards et al., 2012). This is because it does not rely on genomic information and is able to provide several hundred epigenetic fingerprints of a larger number of individuals on a genome-wide scale concurrently, suggesting it could be a powerful detector of differentiation among populations (Bossdorf et al., 2008). Additionally, the MSAP technique is sensitive in that band presence/absence is largely an all-or-nothing response (Blouin et al., 2010). Finally, epigenetic studies of natural populations based on the MSAP technique can detect environmentally induced variation in DNA methylation (Karan et al., 2012; Verhoeven et al., 2010). Thus, the MSAP technique was used to explore the natural variation in our bat populations.

Patterns and levels of genomic methylation in bat populations

The variation in genomic methylation patterns and levels across taxa may have potential functions in evolution (Feng et al., 2010; Sarda et al., 2012; Zemach et al., 2010). Jabbari and colleagues (Jabbari et al., 1997) found that cold-blooded vertebrates, such as fishes and amphibians, have higher CpG methylation levels than warm-blooded animals including mammals and birds, and their later study showed that polar fishes exhibit higher DNA methylation levels than do tropical and temperate fishes (Varriale and Bernardi, 2006), suggesting that the methylation level in vertebrates may be inversely related to their body temperature, thus allowing vertebrates to adapt to their surroundings. Shorter et al. (Shorter et al., 2012) showed that domestic chickens have different methylation levels at specific loci compared with those of their ancestor, the red junglefowl; this may be associated with the long period of domestication. In bats, as in other mammals, DNA methylation is mainly targeted at CpG loci in the genome-wide coverage. The three bat species we studied have similar genome-wide 5′-CCGG-3′ methylation levels (20–27%), but all are below those of other reported animals based on muscle tissues, such as chicken (about 29.4%) (Xu et al., 2007) and swine (about 54%) (Yang et al., 2011), using the MSAP technique. However, the function of lower methylation levels in the evolution of bats requires further investigation.

Epigenetic diversity in bat populations

All three bat species investigated exhibit high levels of epigenetic polymorphisms (Fig. 3). Epigenetic variation is structured into distinct between- and within-population components [β_{ST}=0.316, 0.337 and 0.255, P<0.001, 10^4 permutations for *H. armiger*, *R. pusillus* and *M. fuliginosus*, respectively, using ADE-4 software (Thioulouse et al., 1997)], as is genetic variation. Greater individual epigenotypic variation contributes to the extensive methylation diversity within a population, which could support the ‘raw material’ for adaptive evolution (Herrera and Bazaga, 2010; Kalisz and Purugganan, 2004). Epigenetic diversity of a similar extent to that found in these three bat species has been reported in plants, such as *A. philoxeroides* (Gao et al., 2010), *Hordeum brevisubulatum* (Li et al., 2008) and *Viola cazorlensis* (Jablonka and Lamb, 1998), indicating that extensive intraspecific epigenetic variation and epigenetic structuring may be widespread in wild populations.

Greater epigenetic diversity may have evolutionary potential for wild populations. Latzel et al. (Latzel et al., 2013) found that *Arabidopsis thaliana* plants with diverse epigenetic variation have greater productivity and stability than epigenetically uniform ones. With a varied introduction history, house sparrow (*P. domesticus*)

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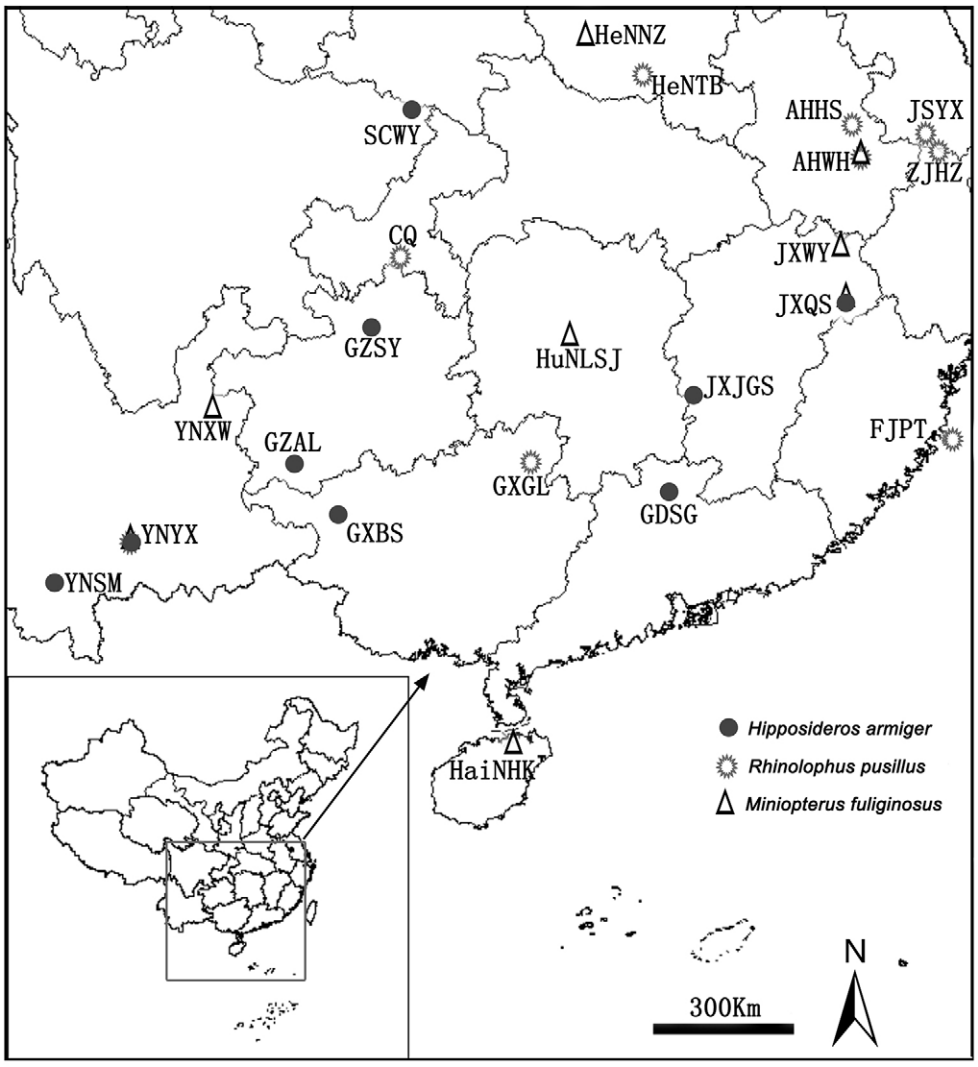

Fig. 2. The study area. The map shows the caves in the sampled localities for the three bat species. For definitions of abbreviations, see Table 1.

populations from Kenya (<50 years) and Florida (~150 years) both showed higher levels of epigenetic variation but different patterns of population methylation, and there was a negative correlation between epigenetic and genetic diversity, suggesting that genome-wide methylation variation may compensate for the decreased genetic variation during introduction (Liebl et al., 2013; Schrey et al., 2012). Populations of the invasive plants Japanese knotweed and alligator weed have low genetic diversity but extensive epigenetic variation, which has facilitated their adaption and spread to new habitats (Gao et al., 2010; Richards et al., 2012). The genomic methylation diversity in bat populations exceeds the corresponding DNA sequence diversity (Fig. 2) and provides a large number of methylation variations, which may be of benefit to bats in adapting to various environments.

Factors influencing epigenetic diversity in bat populations

As Richards (Richards, 2006) reviewed, there are three types of epigenetic methylation: (1) obligatory – epigenetic variation is completely dependent on genetic divergence; (2) facilitated – epigenetic variation is directed or potentiated loosely by genotype; and (3) pure – epigenetic variation, generated by stochastic events or environmental changes, is independent of genetic variation. Thus, DNA sequence divergence, random events or varied environments may contribute to epigenetic diversity alone or together.

Effects of genetic divergence on epigenetic diversity in bat populations

The relationship between epigenetic and genetic diversity is a key aspect in epigenetic research because it determines the degree of phenotypic variation that can be explained by epigenetic effects alone or the significance of meiotic transmission of epigenetic variance (Richards, 2006).

In general, the correlation between the two profiles is estimated by multilocus approaches based on the methylated and non-methylated AFLP fragments for natural populations (Bonin et al., 2007). The lack of, or weak, correlation between the two diversities means epigenetic mechanisms may be independent or at least partly independent from genetic control, as an additional system in evolution (Bossdorf et al., 2008; Bossdorf and Zhang, 2011). For instance, the large variation in DNA methylation in *Arabidopsis thaliana* plants is not correlated with their genetic divergence (Cervera et al., 2002; Vaughn et al., 2007). Epigenetic variation in populations of the southern Spanish violet *V. cazorlensis* has a weak association with herbivory-related genetic variation but is significantly correlated with herbivory stress (Herrera and Bazaga, 2011). In contrast, a significant correlation between epigenetic and genetic profiles has been reported in other plants (Herrera and Bazaga, 2010; Li et al., 2008) and humans (Bjornsson et al., 2008; Liu et al., 2010), which suggests interdependence or position-

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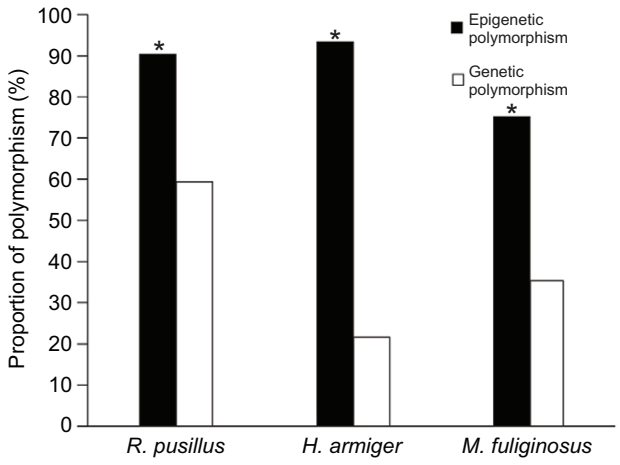

Fig. 3. The difference between epigenetic and genetic polymorphisms in the three bat species. Asterisks indicate a significant difference between epigenetic and genetic polymorphism (P<0.05).

dependent correlations between the two profiles (Chuang et al., 2012; Hellman and Chess, 2010).

We found significant correlation between the two profiles in all three bat species (r=0.299, P=0.048 for *H. armiger*; r=0.524, P=0.010 for *R. pusillus*; r=0.6446, P=0.0245 for *M. fuliginosus*), indicating that epigenetic differentiation may be dependent on, or a downstream consequence of, genetic variation (Liu et al., 2012). Here, we cannot differentiate between the three types of methylation as reviewed by Richards (Richards, 2006). However, the significant relationship suggests that most of the methylation variation among bat populations may belong to the obligatory or facilitated epigenetic variation type, which indicates that correlated genetic–epigenetic variation may be a possible pathway for the evolution of bat populations (Herrera and Bazaga, 2010).

Epigenetic drift or epimutations contribute to epigenetic diversity

The extensive DNA methylation polymorphisms and diversity in bat populations significantly exceed the corresponding differences in genetic profiles (Fig. 3), suggesting that at least a part of methylation variation is independent of genetic divergence or can be identified as pure epigenetic variation. Some of the pure methylation variations may be caused by stochastic events like drift or epimutation. Epigenetic drift could induce the divergence in methylation patterns by errors in the mitotic process (Richards, 2008). Epigenetic drift is also always associated with ageing processes and may have profound effects on physiology (Martin, 2005; West et al., 2013). Random epimutations that are not induced by environmental factors (Robertson and Wolf, 2012) may contribute to the extensive epigenetic diversity among bat populations. These stochastic events may also cause epigenetic and genetic divergence to happen in parallel such that the significant relevance between the two profiles may be a non-functional link (Richards et al., 2010).

Environmental factors contribute to epigenetic diversity

Bats are sensitive to environmental changes (Jones et al., 2009), and populations living in diverse environments exhibit extensive variation in genome-wide DNA methylation. At least some of the epigenetic variance may be induced by environmental variation. This includes meteorological conditions (such as temperature, precipitation), cave size (day roosts), diet and human activities such as cave tourism, illumination and noise pollution surrounding the habitats, all of which could significantly affect the development, distribution and behaviours of bats (Bourne and Hamilton-Smith, 2007; Mann et al., 2002; Ransome and McOwat, 1994; Schaub et al., 2008).

Ecological factors covarying across geographical locations may have a greater impact on DNA methylation of bats (Liu et al., 2012). Different key ecological factors accounted for the epigenetic variation in the three bat species, probably because of their disparate characteristics in many aspects. For example, meteorological conditions may contribute to the epigenetic diversity of *R. pusillus*, while human disturbance may affect the methylation variation in both *H. armiger* and *M. fuliginosus* (S.L. and J.F., unpublished data).

It should be noted that epigenetic cues are more flexible and dynamic than are genetic markers (Richards, 2006), which means that environment-induced methylation patterns in bats may be reversible when the normal environment is restored. Gao and colleagues (Gao et al., 2010) designed a controlled common-garden experiment on the alligator weed *A. philoxeroides* to test the reversibility of methylation patterns. All the experimental plants from terrestrial and aquatic habitats with diverse morphological and epigenetic patterns were planted under the same conditions after tissue culture, and showed similar features in both. Similarly, research on A^{vy} mice showed that the maternal dietary methyl donor similar to genistein compensates for the lower methylation of the six CpG sites in a retrotransposon upstream of the transcription start site of the *Agouti* gene in the fetus, protecting offspring from obesity, which also suggests the reversible features of methylation (Dolinoy et al., 2006).

Environment-induced epigenetic variance may facilitate the persistence of bat populations. In general, we consider that the varied methylation patterns that may occur as a result of changes in the environment could induce the organism to adapt to the environmental change. Thus, those methylation variations may contribute to the extreme extension of an invasive species such as the alligator weed, Japanese knotweed and house sparrow, and even broaden the ecological niche of a flower-living yeast (*Metschnikowia reukaufii*), as reviewed above. Bats living in varied environments, such as areas far from and near to humans, have extensive methylation diversity. Once rapid environmental changes occur, e.g. cave tourism, the epigenetic cues may also facilitate the bats acquiring a new phenotype to overcome the harmful effects from illumination and noise. However, it should be noted that epigenetic variance may have adverse effects on the persistence of bat populations if an error occurs in matching the phenotype with the changed surrounding or if the phenotype persists following a subsequent environmental change (Ledón-Rettig, 2013).

Heritable epigenetic variation in bats and its role in evolution

Heritable epigenetic marks pose a serious challenge to the Modern Evolutionary Synthesis, which assumes that DNA sequence variance is the only heritable variation in natural populations. The greater epigenetic variance existing in individuals (68.4%, 66.3% and 74.5% for *H. armiger*, *R. pusillus* and *M. fuliginosus*, respectively) provides vast potential heritable methylation variations. Environment-induced epigenetic patterns in bats may be inherited by both mitosis and meiosis. Epigenetic patterns in somatic cells are critical in maintaining the physiological stability of the cell and the health of an organism. Somatic epigenetic variation could result in a new phenotype for the individual and may be transmitted between its daughter cells by mitosis (Skinner, 2011). The heritable methylation patterns in the germ line could be inherited by future

generations (Jablonka and Raz, 2009; Richards, 2006), which could reflect the exposure to their parental or ancestral environmental conditions (Dias and Ressler, 2014; Crews et al., 2007; Jensen 2013).

It should be noted that epigenetic reprogramming would occur in mammals during early development of germ cells and pre-implantation embryos, which is crucial for imprinting and establishing nuclear totipotency (Geiman and Robertson, 2002; Reik et al., 2001). The epigenetic patterns, especially environment-induced methylation variations from parents, may be erased and reset during the early development of the offspring. However, not all epigenetic marks are reset in each generation, and some of them may be stably inherited across generations as reported in many plant and animal species (Grant-Downton and Dickinson, 2006; Jablonka and Raz, 2009; Nätt et al., 2012; Richards, 2006). Heritable epigenetic variations that escape resetting provide the insight into the mechanisms of environmental adaption of bats.

Methylation variations may be inherited and maintained stably across generations to ensure they adapt to the changed environments for a long period. However, because of the flexible and dynamic features of epigenetic cues, the phenotypes caused by changed methylation patterns may be 'washed-out' both inter- and intra-generation over several generations, as reviewed in this issue by Burggren (Burggren, 2015). When rapid environmental change occurs again, 'wash-out' may facilitate the offspring to adapt to the new changed surroundings. Thus, epigenetic marks may have a crucial role in the widespread distribution of bat populations.

Conclusions

Bats are key ecological species and reflect wider scale impacts on the biota of interest. They live in diverse habitats with varied phenotypes and are sensitive to environmental changes, such as variable meteorological conditions and anthropogenic effects. Genetic changes could not provide an effective phenotype for bats to adapt to environmental changes rapidly, and it is therefore necessary to consider another explanation. Epigenetic cues, such as DNA methylation, could mediate phenotypic variation in response to environmental changes without genetic divergence, and may provide insight into the mechanisms of environmental adaptation of bat populations.

Here, we have explored population epigenetic diversity in three diverse bat species. Their greater epigenetic polymorphisms and significant epigenetic structures exhibit extensive methylation diversity within and among populations and individuals, as also found in plants, indicating that the extensive individual epigenotypic variations may be evolutionarily significant to wild populations. The genetic divergence, drift or epimutation, and environmental variation contribute to the wide epigenetic diversity. The heritable environment-induced epigenetic variation may be beneficial or harmful for bat populations, and could be one of the driving forces in bat evolution in addition to natural selection and the genetic profile.

It is still very hard to explore the mechanisms behind the processes of epigenetic variation in response to ecological stress of bats by common-garden experiments because of their extensive genetic diversity. In addition, other markers should be considered, such as histone methylation and acetylation. In the future, new and effective high-throughput sequencing techniques could provide further insight into the possible relationships between genetic, epigenetic and phenotypic variation, which could enable us to elucidate the evolutionary potential of environment-induced epigenetic variation in bat populations.

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Competing interests

The authors declare no competing financial interests.

Author contributions

S.L. and J.F. conceived and designed the study. S.L. and K.S. executed the experiments. S.L. wrote the draft. S.L., K.S. and T.J. revised the article.

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