Identification of hybrids between the Japanese giant salamander (Andrias japonicus) and Chinese giant salamander (Andrias davidianus) using deep learning and smartphone images

Kosuke Takaya[1], Takeshi Ise[2], and Yuki Taguchi[3]

[1]Kyoto University Graduate School of Agriculture Faculty of Agriculture
[2]Kyoto University Field Science Education and Research Center
[3]Asa Zoological Park

May 2, 2023

Abstract

Biological invasions are recognized as one of the factors causing biodiversity loss. Incomplete reproductive isolation with a closely related species can result in hybridization when a non-native species is introduced into a new habitat. Management of hybrids is essential for biodiversity conservation; however, the distinction between the two species becomes a challenge in cases of hybrids with similar characteristics to native species. Although image recognition technology can be a powerful tool for identifying hybrids, studies have yet to utilize deep learning approaches. Hence, this study aimed to identify hybrids between native Japanese giant salamanders (Andrias japonicus) and non-native Chinese giant salamanders (Andrias davidianus) using EfficientNet and smartphone images. We used smartphone images of 11 native individuals (with 5 training and 6 test images) and 20 hybrid individuals (with 5 training and 15 test images). In our experimental environment, an AI model constructed with efficientNet-V2 showed 100% accuracy in identifying hybrids. In addition, highlighting the regions that influenced the AI model's predictions using Grad-CAM revealed that salamander head spots are responsible for correctly classifying native and hybrid species. The results of this study revealed that our approach is one of the methods that enable the identification of hybrids, which was previously considered difficult without identification by the experts. Furthermore, since this study achieved high-performance identification using smartphone images, it is expected to be applied to a wide range of low-cost identification using citizen science.

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Kosuke Takaya [*a)], Yuki Taguchi [b)] and Takeshi Ise [c)]

[a)] Graduate School of Agriculture, Kyoto University, Kyoto, Japan

[b)] Hiroshima City Asa Zoological Park, Hiroshima, Japan

[c)] Field Science Education and Research Center, Kyoto University, Kyoto, Japan

*Corresponding author: Kosuke Takaya, Graduate School of Agriculture, Kyoto University, Oiwake, Kitashirakawa Sakyo-ku, Kyoto 606-8502, Japan; takaya.kosuke.25a@st.kyoto-u.ac.jp; TEL: 075-753-6425; FAX: 075-753-6451

Abstract

Biological invasions are recognized as one of the factors causing biodiversity loss. Incomplete reproductive isolation with a closely related species can result in hybridization when a non-native species is introduced into a new habitat. Management of hybrids is essential for biodiversity conservation; however, the distinction between the two species becomes a challenge in cases of hybrids with similar characteristics to native species. Although image recognition technology can be a powerful tool for identifying hybrids, studies have yet to utilize deep learning approaches. Hence, this study aimed to identify hybrids between native Japanese giant salamanders (*Andrias japonicus*) and non-native Chinese giant salamanders (*Andrias davidianus*) using EfficientNet and smartphone images. We used smartphone images of 11 native individuals (with 5 training and 6 test images) and 20 hybrid individuals (with 5 training and 15 test images). In our experimental environment, an AI model constructed with efficientNet-V2 showed 100% accuracy in identifying hybrids. In addition, highlighting the regions that influenced the AI model's predictions using Grad-CAM revealed that salamander head spots are responsible for correctly classifying native and hybrid species. The results of this study revealed that our approach is one of the methods that enable the identification of hybrids, which was previously considered difficult without identification by the experts. Furthermore, since this study achieved high-performance identification using smartphone images, it is expected to be applied to a wide range of low-cost identification using citizen science.

Keywords

Andrias japonicus, **giant salamander, efficientNet-V2, gradient-weighted class activation mapping, hybrid**

Introduction

Biodiversity is essential for human health, well-being, and a stable environment. Although significant efforts have been devoted toward conservation, biodiversity loss remains a global challenge (Johnson et al. 2017). Anthropogenic activities such as urbanization, agricultural intensification, and species exploitation reduce biodiversity, and evidence indicates that species extinction rates are progressing much faster than in the past (Ceballos et al. 2015). In addition, globalization has led to the introduction of various organisms from their native habitats into new environments and the establishment of non-native populations in new areas. These invasive species cause ecosystem impacts such as predation, niche displacement, and introduction of diseases (Doherty et al. 2016; Haubrock et al. 2021; Kortz and Magurran 2019; Scheele et al. 2019). Furthermore, non-native species are recognized as a driver of recent extinctions (Bellard et al. 2016). The impact of non-native species on biodiversity and ecosystems is accelerating, and this trend is expected to continue (Pyšek et al. 2020). Therefore, the mitigation of biological invasions is essential for biodiversity conservation.

When non-native species are introduced into a new habitat, newcomers sometimes encounter close relatives. In such cases, hybridization occurs due to incomplete reproductive isolation from closely related species. Hybridization in non-native species is frequently observed and considered an evolutionary mechanism that determines invasion success (Bock et al. 2021). For example, hybrid fitness is occasionally superior to the parental species; (i.e., hybrid vigor). In addition, hybrids also have intermediate traits or different traits from the parent species, and some traits may determine the establishment success of invasive species (Coulter et al. 2020). For instance, a meta-analysis of plants, animals, and fungi demonstrated that invasive hybrids have a larger body size and are more fecund than their parent species (Hovick and Whitney 2014). Furthermore, early invasive populations are affected by density-dependent processes such as the Allee effect. However, hybridization provides mating partners for invasive species, which could reduce the Allee effect and promote invasions (Yamaguchi et al. 2019).

Hybrids of similar species pose a threat to genetic diversity because introduced alleles may eventually replace the native alleles (Fitzpatrick and Shaffer 2007). Therefore, controlling hybrid species is necessary to conserve biodiversity. However, difficulties in distinguishing between native and hybrid species is a critical issue when trapping hybrids. Hybrids were detected using morphological characteristics until the mid-1960s (Allendorf et al. 2001). This approach assumes that hybrids exhibit intermediate characteristics of their parent species; however, this assumption does not generalize to all cases because they often show a mosaic of parental phenotypes. In addition, morphological characteristics cannot be determined whether an individual is a first-generation or a backcross-generation hybrid. Misidentification of species can also cause conservation problems. For example, inadequate identification of target species could negatively impact native species. In fact, native frogs have been killed in Australia due to misjudgments while removing the non-native cane toad (*Rhinella marina*) (Somaweera et al. 2010).

The development of molecular genetic techniques, such as allozyme electrophoresis and PCR, has overcome these challenges (Allendorf et al. 2001). DNA analysis allows accurate species identification and can reveal individuals' degree of hybridization, which would be difficult to determine using morphological traits. However, these analyses are time-consuming and costly, limiting the quick identification of hybrids and large-scale surveys.

In recent years, deep learning image recognition technology, a novel group of artificial intelligence approaches, has begun to be utilized in both species and individual identification in ecology. Identifying and counting animal species in images provides basic but essential information (Tuia et al. 2022). Many previous studies have combined camera traps and deep learning to identify species. For instance, Norouzzadeh et al. (2018) used 3.2 million images from camera traps in the Serengeti National Park to successfully identify 48 species. In addition, these techniques have been applied to individual identification, such as green turtles (Carter et al. 2014), chimpanzees (Schofield et al. 2019), and brown bears (Clapham et al. 2020). Furthermore, this technology has already been used to detect non-native species (Ashqar and Abu-Naser 2019; Guo et al. 2022; Takaya et al. 2022). Although a similar approach may provide a new method for identifying hybrids in the field, studies have yet to apply deep learning models to identify hybrids.

The Japanese giant salamander (*Andrias japonicus*) is an amphibian endemic to Japan and is threatened with extinction, as its population has declined due to habitat degradation and fragmentation (Tochimoto et al. 2007; Taguchi and Natuhara 2009; Yamasaki et al. 2013). In the 2022 IUCN Red List, the conservation status rank of this species was changed from Near Threatened to Vulnerable (IUCN 2022). One reason for this change is its hybridization with the non-native Chinese giant salamander (*Andrias davidianus*), which is the same genus as *A. japonicus* . The Chinese giant salamander is also threatened with extinction in their original habitat, but individuals introduced to Japan in the early 1970s have become wild, and hybridization with Japanese giant salamanders is an issue. For example, the Kyoto City government survey revealed that only 4 (2%) out of 244 captured individuals were native species, and the remaining were 240 (98%) hybrids and non-native species in the Kamo river basin in Kyoto, requiring rapid action (The Kyoto City Government 2015). However, the number of areas where hybrids were caught is increasing and has already been confirmed in six prefectures in western Japan (Kyoto, Mie, Nara, Shiga, Okayama, and Hiroshima). Since hybrid species have a spot pattern that inherits the characteristics of both native and non-native species, individuals with the potential for hybridization are captured by visual screening and DNA analysis is also applied for accurate identification. While this approach is reliable, identifying hybrids by their spot patterns requires specialized knowledge, and DNA analysis is time-consuming and expensive. If identifying hybrid salamanders from images could work well, it does not need time and cost as DNA analysis. It also facilitates early detection and effective capture of suspected hybrid individuals via citizen science, thereby contributing to the effective conservation of native Japanese giant salamanders.

The first objective of this study is to identify hybrids between Japanese giant salamanders and Chinese giant salamanders from images based on deep learning. Our approach allows the public to photograph and detect hybrid individuals without specialized knowledge. In recent years, citizen science has been adopted to manage invasive species (Larson et al. 2020), and a similar method could be applied to hybrids. The second objective is to clarify which features the AI model uses as criteria to determine hybrids. Spot patterns are difficult to quantify compared to measurable morphological features. However, techniques such as Grad-CAM allow visualization of the important region for the AI model's prediction. If specific essential areas in identifying hybrids can be clarified, that information is valuable for the general public to identify hybrids. Although there is a proposal to divide the Chinese giant salamander into three species (Turvey et al. 2019), our study uses*Andrias davidianus* instead of making this distinction.

Materials and Methods

Image acquisition

In this study, 11 native individuals and 20 hybrid individuals were used (Supplementary Figures 1 and 2). The Hiroshima City Asa Zoological Park, where these individuals are kept, has been researching and trying to breed Japanese giant salamanders since 1971 and is one of the leading facilities in Japan for research and conservation on this species. Native individuals were already kept at the Japanese giant salamander conservation and breeding facility. Hybrids were captured in the wild in Hiroshima Prefecture in May 2022 and were kept in the same facility.

11 native individuals were photographed on August 20, 2022, at 11:00 AM. Each individual was recorded for approximately 30 seconds using an iPhone 11 with a 12-megapixel camera. We photographed the Japanese giant salamanders in the water from above (Supplementary Figure 3). The videos were converted to ten still JPEG images (1920 × 1080) per second using the Free Video to JPG Converter.

20 hybrid individuals were photographed on November 19, 2022, at 2:00 PM using an iPhone SE equipped with a 12-megapixel camera. The method of image collection was the same as for the native individuals.

Ethics declarations

The Law protects Japanese giant salamanders for the Protection of Cultural Properties as a "National Natural Monument." Therefore, this study was approved by the Hiroshima City Asa Zoological Park, which has permission from the Agency for Cultural Affairs and is categorized as a non-invasive study.

Framework

The framework of this study is shown in Figure 1. The salamander heads in the images were automatically detected using YOLOv5 (Redmon et al. 2016) and used as either training or test images. The training data used 5 native and 5 hybrid individuals selected randomly from the two groups (Table 1; Supplementary Figures 1 and 2). The 6 remaining native individuals and 15 remaining hybrid individuals not used in training were selected as test images. These images were resized to 224 x 224 pixels to ensure consistency in size. Additionally, augmentation processing (rotation, crop, brightness, gaussian noise, color jitter, and saturation) was added to the training data to prevent over-fitting. After augmentation, 70% of the images for training and 30% for validation were randomly separated for analysis.

Visualization using Grad-CAM

Deep learning based on convolutional neural networks has achieved high accuracy in different areas of image recognition, such as image classification, object detection, and image segmentation. This high performance is obtained from processing substantial amounts of data with deep neural networks. However, the multilayer, nonlinear structure of deep learning causes difficulty in interpreting the model. This lack of interpretability is a major disadvantage of deep learning, and this technique is sometimes considered a "black box" method. In fact, in fields such as clinical medicine, the lack of interpretability in models is a barrier to the practical application of deep learning (Petch and Nelson 2022). Recently, several approaches have been developed to overcome these challenges. For example, Class Activation Mapping (CAM) provides a heatmap visualization of the regions that influenced the model's predictions, which is valuable information for human interpretation of the results (Zhou et al. 2016). However, this method is not applicable when the Global Average Pooling (GAP) layer is absent, which means it depends on the network structure. Gradient-weighted class activation mapping (Grad-CAM), a generalized version of CAM unconstrained by model structure, has improved this problem (Selvaraju et al. 2017). The algorithm uses class-specific gradient information in the final convolutional layer of a CNN to visualize important regions. This study used Grad-CAM to show visual evidence in classifying native and hybrid species.

EfficientNet-V2

This study trained EfficientNet-V2 to classify images. EfficientNet is a convolutional neural network that scales down the number of layers while scaling down the model (Tan and Le 2019). EfficientNet-V2 is an improved version of EfficientNet with increased training speed and parameter efficiency relative to the previous EfficientNet (Tan and Le 2021). The EfficientNet-V2 model employs neural architecture search (NAS) to optimize model accuracy, size, and training speed. In this study, the EfficientNetV2-B0 model was used as the network, and fine-tuning was performed using a model that had been pre-trained with the Imagenet21k data set. The number of epochs was set to 50, and the batch size was set to 32 for training. Adam was used as the optimization algorithm (optimizer), and dropout was set to 0.3. We employed early stopping to prevent overfitting. Automatic termination was performed when validation loss did not improve more than 0.001 for five consecutive epochs, and we used the weights when validation loss was the best. These analyses were performed using the NVIDIA DGX Station A100. Finally, overall accuracy was used for evaluation.

Results

Classification of native and hybrid species

The 6 native individuals and 15 hybrid individuals used in the test (1,881 images) were all correctly classified, with an accuracy of 100% in our experimental environment. The classification results are presented in the confusion matrix (Figure 2). The vertical axis is the ground truth, and the horizontal axis is the model's prediction. The number in each cell indicates the number of images classified as native or hybrid, and the color of each cell indicates the percentage of images per ground truth. For example, pale blue indicates a ratio of 0.0, indicating that no image is classified for that cell. In contrast, dark blue indicates a ratio of 1.0, indicating that all images were classified for that cell.

Visualization using Grad-CAM

The original images of the native and hybrid species, and the activation maps visualized by Grad-CAM, are shown in Figure 3 and Figure 4. Other individuals used in the analysis are shown in Supplementary Figures 4 and 5. The red indicates the regions the model considers when outputting the prediction results. The results show that the head spots of salamanders are a key area for the classification of native and hybrid species. For native species, the visualized figures indicated that the network learns to recognize relatively distinctive and large black spots. On the other hand, for hybrids, the visualization indicated that the network learned to focus on pale and ambiguous wide regions rather than distinctive and large black spots. However, because the spot pattern differs from individual to individual, the results of Grad-CAM visualization also differ from individual to individual (Supplementary Figures 4 and 5).

Discussion

In this study, we identified hybrids between native Japanese giant salamanders and non-native Chinese giant salamanders from images using deep learning. Historically, visual screening by experts and DNA analysis were applied to identify hybrids. However, the scarcity of experts and the time and cost of DNA analysis were barriers to effective screening. Therefore, we have proposed a novel approach to identifying hybrids using an image recognition technique. A total of 6 native and 15 hybrid individuals were used, and all were correctly classified by the AI model with an accuracy of 100% in our experimental setting. Furthermore, highlighted regions that affect the AI model's prediction suggested that the model distinguished between native and hybrid species based on spot patterns. Although deep learning has already been applied to species and individual identification, this is the first study we know that identifies hybrid species.

EfficientNet-V2 demonstrated that head spot patterns could be used to identify native and hybrid species. One reason for successfully classifying all individuals is the quality of the training and test images. In this study, photographs were taken from a short distance; thus, the high accuracy can be attributed to the clear spot patterns in the images. Another reason is that the salamanders' heads were photographed from a similar angle (Supplementary Figure 3). For example, previous studies have shown that different photo angles reduce identification accuracy (Arzoumanian et al. 2005). In this research, we photographed all individuals from directly above. These images used for training and testing facilitated the comparison of spot patterns and ensured highly accurate results. Training and test images obtained on the same day could also have influenced the high performance. In the future, our approach performance should be carefully evaluated in a varied environment, using images from different dates and locations, before implementing this technology in the field.

Visualized distribution of the heat maps was different for native and hybrid species. For the native species, the model focused on distinctive and large black spots, while for the hybrids, it focused on the pale and ambiguous wide region. These results suggest that the differing spot patterns between native and hybrid species can be utilized for classification. In general, native species have distinctive and large black spots (Supplementary Figure 1), whereas the spots of hybrids are more indistinct than those of native species (Supplementary Figure 2). Experts use these spot pattern differences as one of the criteria to identify hybrid individuals. The results of this study revealed that deep learning would distinguish between native and hybrid species using the same pattern recognition as experts. The heat map could be used as an instruction guide for the general public on hybrid identification because the highlighted graphical figures are visually comprehensible.

Although our approach has achieved high accuracy in identifying native species and hybrids in this study, several challenges still exist. Firstly, we did not consider the hybridization degree, which affects the spot pattern in hybrids. The hybrid captured in Hiroshima used in this study was found in the river recently, which suggests that the generation is less advanced. Since hybrid individuals between Japanese and Chinese giant salamanders are fertile, the spot pattern varied depending on several factors, such as generation. Future work should examine the relationship between the degree of genetic introgression and identification accuracy. Secondly, combining this method with DNA analysis is essential because deep learning-based identification has limitations. For example, due to hybridization, some hybrids have previously been observed with spots indistinguishable from those of Japanese giant salamanders. DNA analysis is the only method to determine the species in such cases. Therefore, our technology could be applied for the early detection of suspected hybrids through citizen science and quick identification by computer vision. In addition, advanced research might allow the identification of backcrossed hybrids that are difficult to distinguish even for experts because spots are extremely close to native species. Finally, this study was conducted in the daytime in uniform photographic conditions. Giant salamanders must be photographed under lights in field-based surveys because they are nocturnal. In the future, it is necessary to determine whether images obtained under various light conditions could be used to identify hybrids.

Hybridization between native and invasive species is one of the major causes of biodiversity loss (Bourret et al. 2022). However, detecting hybrids was challenging when the hybrids were similar to the native species. Deep learning image recognition techniques can be a valuable tool to support the visual identification of hybrids. We proposed a new approach for classifying native species and hybrids using smartphone images that could be utilized in citizen science. Hybrid identification based on spot patterns has previously been difficult and thus limited to experts; however, artificial intelligence analysis allows the public to detect hybrids easily. In particular, the distribution of hybrids is expanding, meaning that managing hybrids is a priority task for conserving Japanese giant salamanders. The findings of this study can potentially prevent the future spread of hybrids by providing a method for the efficient discovery of these individuals.

Conclusion

In this study, we applied deep learning to identify hybrids between Japanese and Chinese giant salamanders. Our results show that the head of giant salamanders is effective for classification. The use of Grad-CAM also revealed that the spot pattern is important for identifying the two species. Visual identification of hybrids has historically been restricted to specialists, but our approach could give a possibility for the public to identify hybrids. These results support the identification of hybrids, especially within the context of citizen science.

Acknowledgements

We are very grateful to the Hiroshima City Asa Zoological Park for their cooperation in our research on Japanese giant salamanders. Under permission from the Agency for Cultural Affairs, the Asa Zoological Park is researching and breeding the Japanese giant salamander, a nationally protected species. This work was supported by the Sasakawa Scientific Research Grant from The Japan Science Society.

Author contributions

Kosuke Takaya: Conceptualization, data analysis, interpretation, and preparation of the first original manuscript

Yuki Taguchi: Conceptualization, data collection, interpretation, and suggestions for the original manuscript

Takeshi Ise: Guided all steps of the analysis and manuscript preparation.

Competing interests

The authors declare no conflicts of interest associated with this manuscript.

Data availability statement

The code and models are both also archived at Zenodo.

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