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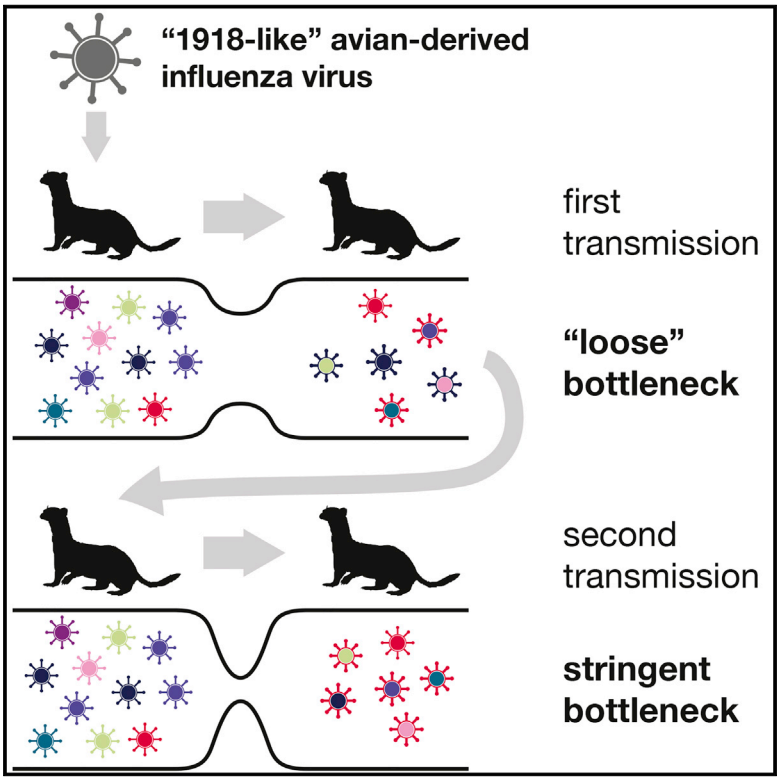
Selective Bottlenecks Shape Evolutionary Pathways Taken during Mammalian Adaptation of a 1918-like Avian Influenza Virus

Graphical Abstract



Highlights

- HA diversification arising during initial ferret adaption of avian flu virus is maintained
- Low-frequency transmissible polymerase variants arise subsequently
- Transmission bottleneck selects specific HA variants
- Mammalian transmission can evolve through multiple genetic pathways

Authors

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In Brief

Human pandemic influenza viruses can emerge unpredictably from avian reservoirs. Moncla et al. trace the evolutionary path of an avian-like H1N1 virus as it adapts to mammals. They find that initial adaption involves a loose bottleneck, which becomes selective as adaptation progresses, with mammalian transmission evolving via multiple genetic pathways.

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Selective Bottlenecks Shape Evolutionary Pathways Taken during Mammalian Adaptation of a 1918-like Avian Influenza Virus

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SUMMARY

Avian influenza virus reassortants resembling the 1918 human pandemic virus can become transmissible among mammals by acquiring mutations in hemagglutinin (HA) and polymerase. Using the ferret model, we trace the evolutionary pathway by which an avian-like virus evolves the capacity for mammalian replication and airborne transmission. During initial infection, within-host HA diversity increased drastically. Then, airborne transmission fixed two polymerase mutations that do not confer a detectable replication advantage. In later transmissions, selection fixed advantageous HA1 variants. Transmission initially involved a "loose" bottleneck, which became strongly selective after additional HA mutations emerged. The stringency and evolutionary forces governing between-host bottlenecks may therefore change throughout host adaptation. Mutations occurred in multiple combinations in transmitted viruses, suggesting that mammalian transmissibility can evolve through multiple genetic pathways despite phenotypic constraints. Our data provide a glimpse into avian influenza virus adaptation in mammals, with broad implications for surveillance on potentially zoonotic viruses.

INTRODUCTION

In 1918, a novel H1N1 influenza virus, at least partially avian in origin, emerged and killed ~50 million people worldwide (Taubenberger et al., 2001, 2005; Vana and Westover, 2008; Worobey et al., 2014). Influenza viruses can quickly generate within-host genetic diversity due to their high mutation rates, rapid replication, and large population sizes, facilitating adaptation to new environments and host species (Baccam et al., 2006; Drake and Holland, 1999; Lauring and Andino, 2010; Nobusawa and Sato, 2006; Parvin et al., 1986; Sanjuán et al., 2010). Defining how natural selection and genetic bottlenecks shape avian influenza virus adaptation to mammals is critical for understanding how pandemics occur and identifying potentially zoonotic viruses in nature.

Transmission bottlenecks can define subsequent viral evolution. We showed that positive selection for specific hemagglutinin (HA) variants caused a strong bottleneck during evolution of ferret-transmissible reassortant H5N1 viruses (incorporating avian virus HA in a human H1N1 virus backbone) (Wilker et al., 2013). Another study showed that founder effects, random nonselective processes, drive bottlenecks during mammal-adapted seasonal influenza virus transmission in ferrets and guinea pigs (Varble et al., 2014). The evolutionary forces acting as fully avian-derived influenza viruses evolve toward mammalian transmission have not been assessed.

Recently, members of our team (Watanabe et al., 2014) examined the potential for a virus resembling the 1918 strain to re-emerge from wild bird reservoirs by generating an avian-derived reassortant virus with close sequence identity to the 1918 pandemic virus. In ferret passage, this "1918-like" virus acquired substitutions in HA and polymerase associated with altered tissue tropism, enhanced replication, and efficient transmissibility (Watanabe et al., 2014). Here, we use deep sequencing to evaluate the evolutionary processes by which these viruses adapted to replication and transmission in mammals. No new transmission experiments were conducted for the present work, and all ferret nasal swabs were collected prior to the gain-of-function research pause.

RESULTS

Transmissible HA Variants Arise in Ferrets

1918-like avian viral replication was limited in directly inoculated animals, and neither virus nor specific antibodies were detected in contact ferrets (Watanabe et al., 2014). Introducing the mammal-adapting substitution PB2 E627K enhanced replication (Figures S1A, S1B, and S1E), but not transmission. To assess the impact of known mammalian-adapting mutations in HA on transmission of this virus, HA E190D and G225D (H3, mature peptide numbering) were introduced into the 1918-like avian/PB2 E627K virus, generating what we will term the "avian-like" "HA190D225D" virus. Deep sequencing showed that both HA